



Vitamin D Status and TB Treatment Outcomes in Adult Tanzanian Patients

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Vitamin D Status and TB Treatment Outcomes in Adult Tanzanian Patients

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3 1 *Abstract*
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5 2 **Objectives:** Vitamin D is an immunomodulator and can alter response to tuberculosis
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8 3 treatment, though randomized trials have been inconclusive to date. We present the first
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10 4 comprehensive analysis of the associations between vitamin D status and TB treatment,
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12 5 T-cell counts, and nutritional outcomes by HIV status.
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17 7 **Design:** Cohort study
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22 9 **Setting:** Outpatient clinics in Dar es Salaam, Tanzania
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27 11 **Participants:** 25-hydroxyvitamin D levels were assessed in a cohort of 677 patients with
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29 12 TB (344 HIV-infected) initiating anti-TB treatment at enrollment in a multivitamin
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31 13 supplementation (excluding vitamin D) trial.
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36 15 **Primary and secondary outcome measures:** Information on treatment outcomes such as
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38 16 failure and relapse, HIV disease progression, T-cell counts, and anthropometry was
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40 17 collected routinely, with a median follow-up of 52 and 30 months for HIV-uninfected and
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42 18 HIV-infected patients, respectively. Cox and binomial regression, and generalized
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44 19 estimating equations were used to assess the association of vitamin D status with these
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46 20 outcomes.
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52 22 **Results:** Mean vitamin D concentrations at enrollment were 69.8 (\pm 21.5) nmol/L [27.9
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54 23 (\pm 8.6) ng/mL]. Vitamin D insufficiency ($<$ 75 nmol/L) was associated with a 66% higher
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3 24 risk of relapse (95% CI: 4%, 164%; 133% higher risk in HIV-uninfected patients). Each
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5 25 unit higher vitamin D levels at baseline were associated with a decrease of 3 (p=0.004)
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8 26 CD8 and 3 (p=0.01) CD3 T-cells/ μ L during follow-up in HIV-infected patients. Low
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10 27 vitamin D was also associated with a greater decrease of BMI (-0.21 kg/m²; 95% CI:-
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12 28 0.39, -0.02), during the first eight months of follow-up. No association was observed for
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15 29 vitamin D status with mortality or HIV disease progression.
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19
20 31 **Conclusions:** Adequate vitamin D status is associated with a lower risk of relapse and
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22 32 with improved nutritional indicators such as BMI in TB patients, with or without HIV
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24 33 infection. Further research is needed to determine the optimal dose of vitamin D and
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26 34 effectiveness of daily vitamin D supplementation among patients with TB.
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4 37 **Article Focus:** Recent laboratory data has suggested that optimal vitamin D status may
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6 38 be associated with a more effective immune response to TB infection, a faster rate of
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8 39 bacteriologic cure, and better long-term outcomes. However, clinical and epidemiological
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10 40 studies have found inconsistent results. In this paper, we present the first comprehensive
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12 41 analysis of the associations between vitamin D status and TB treatment, T-cell counts,
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14 42 and nutritional outcomes by HIV status.
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20 44 **Key Messages:** We found that patients with adequate vitamin D status were less likely to
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22 45 experience a relapse during follow-up after completing TB treatment. They were also
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24 46 more likely to have a better nutritional status, as assessed by their body mass index,
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26 47 during follow-up, compared to patients with low vitamin D status. The results provide
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28 48 justification for conducting both a dose response study to determine optimal dose of
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30 49 vitamin D and a randomized controlled trial of daily vitamin D supplementation among
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32 50 patients with TB.
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39 52 **Strengths and Limitations of this study:** The major strengths of this study include a
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41 53 large number of participants, more than half of whom were HIV-infected, comprehensive
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43 54 assessment of clinical, immunological, socio-demographic, and nutritional parameters,
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45 55 and a long duration of follow-up. On the other hand, the major limitation is the possibility
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47 56 of reverse causation and residual confounding. We have attempted to minimize this
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49 57 through rigorous analyses and adjusting for several potential confounders, including
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51 58 hemoglobin concentrations, HIV status, viral load, CD4 T-cells, and Karnofsky score, in
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53 59 most analyses.
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3 60 **Introduction**
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5 61 *Mycobacterium tuberculosis* is one of the most pernicious infectious diseases and
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8 62 successful pathogens known to man. More than 95% of the estimated cases and deaths
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10 63 due to tuberculosis (TB) occur in low-income countries. The United Republic of
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12 64 Tanzania is one of the 22 high-burden countries that account for 80% of global TB cases.
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14 65 Tanzania has an incidence of 177 cases per 100,000 population per year and a prevalence
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16 66 of 183 cases per 100,000 population per year [1].
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22 68 The spread of Human Immunodeficiency Virus (HIV) has fuelled the resurgence of the
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24 69 TB epidemic in Tanzania, as in other parts of sub-Saharan Africa [2]. HIV is the
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26 70 strongest factor in the development of active TB; it is estimated that only one out of ten
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28 71 immunocompetent persons infected with TB develops active TB in his/her lifetime;
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30 72 whereas, one out of ten HIV-infected persons infected with TB will develop active TB
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32 73 every year. An estimated 38% of TB patients in Tanzania are also infected with HIV [1].
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38 75 Current treatment regimens, given under appropriate management conditions, are nearly
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40 76 100% curative for patients with drug-susceptible organisms. However, in Tanzania,
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42 77 treatment fails in 12-17% of the cases. Additionally, TB patients in settings such as
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44 78 Tanzania grapple with multiple health-related and quality of life issues, which are not
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46 79 addressed adequately with treatment alone.
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52 81 Recent data has suggested that optimal vitamin D status may be associated with a more
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54 82 effective immune response to TB infection, a faster rate of bacteriologic cure, and better
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3 83 long-term outcomes. For example, a recent cross-sectional study found that vitamin D
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5 84 deficiency is highly prevalent in South Africa and is associated with susceptibility to
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8 85 active TB both in the presence and absence of HIV infection [3]. A few randomized trials
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10 86 have also been conducted; two of the recent ones failed to find an effect of vitamin D
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12 87 supplementation on treatment success [4 5]. However, the dose used and duration of
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14 88 supplementation may have precluded finding an effect. Further, most studies had small
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16 89 sample sizes and assessed only a limited number of covariates.
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22 91 In this manuscript, we comprehensively examined the hypotheses that vitamin D status
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24 92 may be associated with response to treatment, risk of treatment failure, laboratory
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26 93 parameters such as T-cell counts, and anthropometric measurements in the context of a
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28 94 randomized trial of micronutrient supplementation (supplement did not contain vitamin
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30 95 D) in Tanzania to better inform future studies or trials.
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36 97 *Materials and Methods*

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38 98 Study Population: The study population and recruitment methods have been described in
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40 99 detail earlier [6]. Briefly, 887 adults with pulmonary tuberculosis (PTB) were enrolled in
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42 100 a randomized trial to examine the effects of micronutrient supplementation on TB
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44 101 treatment failure, relapse, and mortality. The trial started in April 2000 in Dar es Salaam,
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46 102 Tanzania and continued until April 2005. The eligibility criteria for the study included
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48 103 positive sputum smears for acid-fast bacilli (AFB), age between 18 and 65 years,
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50 104 Karnofsky performance score of $\geq 40\%$ [7], plan to stay in Dar es Salaam for 2 years, not
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52 105 being pregnant, and not having received anti-TB treatment during the previous one year.
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3 106 Consenting subjects were randomly assigned in computer-generated permuted blocks of
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5 107 20, stratified by HIV status, to receive a daily oral dose of 1 of 2 regimens:
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7 108 micronutrients (5000 IU of retinol, 20 mg of vitamin B1, 20mg of vitamin B2, 25mg of
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9 109 vitamin B₆, 100 mg of niacin, 50 µg of vitamin B₁₂, 500 mg of vitamin C, 200 mg of
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11 110 vitamin E, 0.8 mg of folic acid, and 100 µg of selenium) or placebo. These doses
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13 111 represent between 6 and 10 times the recommended dietary allowance (RDA) and were
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15 112 being tested at the time among HIV-infected adults from this setting [8]. We chose
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17 113 multiples of the RDA because previous observational studies suggested that HIV-infected
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19 114 individuals need higher dietary intakes of micronutrients to achieve normal serum
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21 115 concentrations [9]. All patients received a daily combination of rifampicin, isoniazid,
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23 116 pyrazinamide, and ethambutol under direct observation of a health worker during the first
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25 117 2 months (intensive phase) followed by 6 months of self-administered daily isoniazid and
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27 118 ethambutol, as per the Tanzania National TB and Leprosy Programme guidelines. None
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29 119 of the HIV-infected patients received antiretroviral therapy, as these medications were
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31 120 not routinely available in Tanzania at the time this trial was conducted.
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35 122 At the time of randomization, research nurses collected information on various socio-
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37 123 demographic characteristics including age, education levels, marital status, and
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39 124 socioeconomic status. Anthropometric measurements were also obtained using
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41 125 standardized procedures [10] at the randomization visit as well as during each monthly
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43 126 follow-up visit. Height was measured to the nearest 0.1 cm using SECA Bodymeter 206
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45 127 stadiometers, weight to the nearest 100 g with SECA 700 balance beam scales, and left
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3 128 mid-upper arm circumference (MUAC) at the midpoint between the acromion and
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5 129 olecranon to the nearest 0.1 cm using non-stretchable tailor's tapes.
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10 131 Physician visits were scheduled every 3 months. During these visits, study physicians
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12 132 inquired about the health of the subject during the preceding period and performed a
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14 133 complete physical examination. The stage of HIV disease was assessed according to the
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16 134 World Health Organization system [11].
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21 136 Ethics Approval: A written informed consent was obtained from all the study
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23 137 participants. The institutional review boards of the Muhimbili University of Health and
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25 138 Allied Sciences, the Tanzanian National AIDS Control Program, and the Harvard School
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27 139 of Public Health approved the study protocol.
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32 141 Laboratory Methods: At the time of initiation of anti-TB treatment, HIV status was
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34 142 assessed among consenting patients using 2 sequential ELISAs (Wellcozyme, Murex
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36 143 Biotech; Enzygnost anti-HIV1/2 Plus, Dade Behring); discrepant results were resolved by
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38 144 Western Blot test (Bio-rad, Genetic Systems). Both pre-test and post-test counseling was
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40 145 provided. A blood sample also was obtained for measurement of hemoglobin and
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42 146 albumin concentrations using AcT Diff II hematology analyzer (Beckman Coulter,
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44 147 Miami) and Hitachi 911 analyzer (Roche Diagnostics), respectively. CD4, CD3, and CD8
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46 148 T-cell counts were determined using FACScout or FACSCan systems (Becton
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48 149 Dickinson, CA, USA). Viral load was also determined using the Amplicor HIV-1
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50 150 monitor v1.5 assay (Roche Molecular Systems, Branchburg, NJ, USA).
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6 152 Assessment of Vitamin D Status: Serum 25-hydroxyvitamin D concentrations were
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8 153 measured using liquid chromatography-mass spectrometry at the Children's Hospital in
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10 154 Boston only at enrollment. We defined low vitamin D status as serum 25(OH)D levels of
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12 155 less than 75 nmol/L and adequate otherwise. Vitamin D deficiency was defined as serum
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14 156 25(OH)D levels of less than 50 nmol/L.
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20 158 Statistical Analysis: We examined the association of vitamin D status with TB treatment
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22 159 outcomes as well as nutritional, immunological, and clinical end points in the entire
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24 160 cohort and separately by HIV status at baseline. TB-related end points included treatment
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26 161 failure, early relapse, and late relapse. Treatment failure by 1 month was defined as
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28 162 positive AFB cultures at 1 month from the initiation of treatment. Relapses were deemed
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30 163 to have occurred in patients with positive cultures, among those who had become culture
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32 164 negative after treatment initiation. Relapses/recurrences included both endogenous
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34 165 reactivation and exogenous reinfection, which could not be distinguished in this study.
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36 166 We calculated the relative risks (RRs) and 95% confidence intervals (CIs) for each of
37
38 167 these outcomes by vitamin D status using binomial regression. We used Cox proportional
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40 168 hazards models to assess the association of vitamin D status with mortality in all patients
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42 169 and HIV disease progression from stage 3 to 4 in HIV-infected participants. We defined
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44 170 the end of follow-up as the date when HIV stage was last assessed.
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53 172 We examined the association of vitamin D status with CD4, CD8, and CD3 T-cell counts,
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55 173 viral load (in HIV-infected participants), indicators of nutritional status (body mass index
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3 174 [BMI] and albumin concentrations), and hemoglobin concentrations using generalized
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5 175 estimating equations (GEEs). These models do not require that all patients have the same
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8 176 number of follow-up assessments or that the follow-up measurements be obtained at
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10 177 exactly the same time points. We assumed a standard normal distribution for repeated
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12 178 continuous end points (T cell subsets, log₁₀ viral load, anthropometry, and albumin and
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14 179 hemoglobin concentrations) and estimated average differences during follow-up by
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16 180 vitamin D status. We used an exchangeable correlation structure to account for within-
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18 181 subject correlations and adjusted the models for the follow-up time when the
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20 182 measurements had been obtained and for the baseline values.
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27 184 We analyzed the data for the entire period and for the first 8 months, coinciding with the
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29 185 expected end of TB treatment. Multivariate analyses adjusted for age, Karnofsky score,
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31 186 baseline hemoglobin concentrations, viral load, HIV status, CD4 T-cell counts, and
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33 187 micronutrient supplementation, unless otherwise specified in the results section or the
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35 188 tables. All analyses were performed using SAS software version 9.3 (SAS Institute Inc.,
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37 189 Cary NC).
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44 **Results**

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46 192 Baseline vitamin D concentrations were available for 677 patients out of the original
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48 193 cohort of 887. Mean 25-hydroxyvitamin D concentration was 69.8 (±21.5) nmol/L [27.9
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50 194 (±8.6) ng/mL] and its distribution is shown in Figure 1. The baseline characteristics of
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52 195 these 677 patients by HIV status are presented in Table 1. 36% of the HIV-infected
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55 196 patients had CD4 T-cell counts below 200 cells/μL. The mean body mass index (BMI)
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3 197 was 19.1 ± 2.7 kg/m². The median follow-up time for HIV-uninfected patients was 52
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5 198 months (inter-quartile range [IQR]: 47-57 months) and for HIV-infected patients was 30
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8 199 months (IQR: 15-41 months).
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13 201 The mean vitamin D concentrations were significantly different across season of blood
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15 202 draw in this cohort ($p=0.004$). Tanzania has four seasons: dry (January-February); long
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17 203 rains (March-June); dry (July-October); short rains (November-December). The boxplot
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19 204 of vitamin D's association with season of blood draw is presented in Figure 2. In
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21 205 subgroup analyses, this association was only observed among the HIV-uninfected
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23 206 patients and not the HIV-infected patients.
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29 208 We examined the correlates of low vitamin D status, defined as serum 25(OH)D
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31 209 concentrations below 75 nmol/L (75 nmol/L) in Supplemental Tables 1 (HIV-uninfected)
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33 210 and 2 (HIV-infected). All factors that had univariate associations with $p<0.20$ were
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35 211 included in a multivariate model; only the factors that had $p<0.05$ were retained in the
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37 212 final model. Among the HIV-uninfected subset, patients enrolled in the dry winter season
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39 213 between July and October were 50% more likely to have low vitamin D concentrations,
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41 214 compared to patients enrolled in the dry summer season between January and February (p
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43 215 for season=0.002). Similarly, the participants with the lowest height were more likely to
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45 216 have low vitamin D status ($p=0.01$). Finally, greater expenditure on food per person per
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47 217 day was associated with a lower risk of having inadequate vitamin D status (Risk Ratio
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49 218 [RR] per 1000 Tanzanian Shillings [approximately 1 US Dollar at the time of the study]:
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51 219 0.76; 95% Confidence Interval [CI]: 0.59, 0.98). In the HIV-infected subset, patients with
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3 220 higher hemoglobin concentrations at baseline were less likely to have low vitamin D
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5 221 status, with a 7% lower risk per 1 g/dL higher hemoglobin level ($p=0.007$). On the other
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8 222 hand, higher number of CD4 T-cells was associated with a higher risk of having
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10 223 inadequate vitamin D status (4% higher risk per 100 CD4 T-cells/ μL ; $p=0.02$).
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15 225 There was no significant association of vitamin D status at TB treatment initiation with
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17 226 mortality or HIV disease progression in this cohort (Table 2). There was no association
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20 227 observed between vitamin D status and treatment failure one month after initiation of TB
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22 228 treatment (Table 3). However, patients with low vitamin D status (<75 nmol/L) had a
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24 229 66% higher risk of relapse after becoming culture-negative at one month after initiation
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27 230 of TB treatment (95% CI: 4%, 164%). This association was more pronounced in those
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29 231 who were not HIV-infected at enrollment in the study (RR: 2.33; 95% CI: 1.26, 4.29). In
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31 232 analysis with continuous vitamin D levels, each nmol/L increase was associated with a
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33 233 1% lower risk of relapse during follow-up ($p=0.04$).
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39 235 Low vitamin D status was observed to have no association with CD4 T-cell counts during
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41 236 the entire follow-up in either the HIV-infected or the HIV-uninfected subsets (Table 4).
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43 237 However, low vitamin D status was associated with greater CD4 T-cell counts during the
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46 238 first eight months of follow-up in the HIV-infected patients (mean difference: 58; 95%
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48 239 CI: 13, 104).
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53 241 In analysis among HIV-infected patients with continuous vitamin D levels, each nmol/L
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55 242 higher vitamin D concentration was associated with a decrease of 3 CD8 and 3 CD3 T-
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3 243 cells per μL . Low vitamin D status was associated with an average of 85 higher CD8 T-
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5 244 cells/ μL during follow-up (95% CI: 4, 165). Similar results were observed when we
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8 245 restricted the analyses to the first eight months of follow-up, the duration of TB treatment
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10 246 at the time of the study in Tanzania. No relationship was observed with mean viral loads
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12 247 during follow-up in the patients who were HIV-infected at the time of enrollment.
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17 249 In analysis examining association of vitamin D status with nutritional parameters in the
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19 250 entire period of follow-up, no significant relationship was observed with BMI, albumin,
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21 251 or hemoglobin concentrations (Table 5). During the first eight months of follow-up,
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23 252 patients with low vitamin D experienced a decline in BMI (Mean: -0.21 kg/m^2 ; 95% CI: -
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25 253 $0.39, -0.02$), compared to patients with adequate vitamin D status. These results were
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27 254 more pronounced in HIV-uninfected patients (Mean: -0.34 ; 95% CI: $-0.60, -0.09$) and not
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29 255 significant in HIV-infected patients. HIV-infected patients with low vitamin D status had
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31 256 increased albumin levels (Mean: 0.94 ; 95% CI: $0.55, 1.32$) during the first eight months
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33 257 of follow-up compared to patients with adequate vitamin D status.
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41 259 *Discussion*

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43 260 In this study among 677 patients with tuberculosis in Tanzania, more than 61% of the
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45 261 participants had vitamin D concentrations below 75 nmol/L (75 nmol/L). Vitamin D
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47 262 concentrations were associated with the season of blood draw, money spent on food per
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49 263 person per day, and height in HIV-uninfected participants and hemoglobin concentrations
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51 264 and CD4 T-cell counts among HIV-infected patients. Low vitamin D status ($<75 \text{ nmol/L}$)
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53 265 was not associated with mortality, HIV disease progression, or treatment failure during
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3 266 follow-up in the entire cohort. However, patients with low vitamin D status had an
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5 267 increased risk of experiencing TB relapse during follow-up. Further, low vitamin D status
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7 268 was associated with a decline in CD8 and CD3 T-cells in both the first eight months (the
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9 269 duration of TB treatment) and the entire period of follow-up. A similar relationship was
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11 270 observed with BMI in the first eight months of follow-up.
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17 272 Our study was conducted in Dar es Salaam, the largest urban center in Tanzania, and just
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19 273 six degrees south of the Equator. The prevalence of low vitamin D status (>61%) in this
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21 274 study is higher than the approximately 40% found in a previous study among TB patients
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23 275 in Mwanza, Tanzania [12] and in our studies among HIV-infected pregnant women
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25 276 (~85% of them had stage 1 HIV disease, unlike this study) in Dar es Salaam [13 14].
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27 277 However, this prevalence is lower than what was observed in a cross-sectional study in
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29 278 South Africa, where 88% of HIV-uninfected and 97% of HIV-infected TB patients had
30
31 279 low vitamin D status. The mean vitamin D concentration in this study was 69.8 nmol/L,
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33 280 compared to 86.5 nmol/L in the study in Mwanza and 28.8-40 nmol/L in the South
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35 281 African study. One study from Thailand observed similar levels (69.0 nmol/L) in TB
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37 282 patients [15]; Thailand is located at a similar distance from the Equator as Tanzania,
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39 283 though it is in the northern hemisphere. Similar to the study in South Africa, the vitamin
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41 284 D levels were lowest in our study in the dry winter season between July and October,
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43 285 though the differences were not as stark. For example, the mean vitamin D concentration
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45 286 in January to March in the South African study was 56.8 nmol/L and 30.8 nmol/L
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47 287 between July and September, whereas in our study, the concentrations were 74.8 nmol/L
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49 288 for January through February, and 66.3 nmol/L for July through October.
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5 290 Vitamin D is synthesized in the skin through the action of ultraviolet light on 7-
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8 291 dehydrocholesterol. Fatty fish, such as salmon and sardines, are good sources of vitamin
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10 292 D in the diet but are not widely available everywhere and are usually expensive.
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12 293 Increasing urbanization and a tendency to spend most time indoors are major factors that
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14 294 contribute to the inability of the skin to synthesize adequate amounts of vitamin D [16-
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17 295 18]. Additionally, the TB disease itself and/or the HIV co-infection in the participants in
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19 296 this study are probably the primary reasons for restricted physical activity, lack of
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21 297 adequate exposure to sunlight, and consequent low concentrations of vitamin D.
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27 299 Several other investigators have examined correlates of vitamin D status in TB patients.
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29 300 The study in Mwanza found that marital status, BMI, and serum transferrin receptor
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31 301 concentrations were correlated with vitamin D status. Though the first two were
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33 302 correlated with vitamin D status in our study in univariate analyses, neither remained
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35 303 significant in multivariate analyses. We didn't measure serum transferrin receptor in our
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37 304 study, though we did observe a correlation of vitamin D status with hemoglobin
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39 305 concentrations among the HIV-infected subset. Another study in South Africa found that
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41 306 TB status (active disease vs. latent infection), month of sampling, and BMI were
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43 307 significantly correlated with vitamin D status in multivariate analyses [3]. All patients in
44
45 308 our study had active disease, and we didn't observe a relationship with BMI in our
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47 309 analyses. The study in South Africa incorporated only those correlates associated with
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49 310 serum 25(OH)D concentration with $P < 0.05$ in univariate analysis in the multivariate
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51 311 model. This may have precluded selection of important covariates and confounders, if
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3 312 measured, and produced biased estimates and confidence intervals; increasing the
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5 313 nominal significance level to 20% or more, as used in this study [19] can eliminate most
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7 314 of this bias. Most other studies have been with smaller sample sizes and have examined a
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9 315 limited set of covariates, compared to the current study.
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15 317 There was no association of vitamin D status with mortality or HIV disease progression
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17 318 in this cohort, unlike our previous studies among HIV-infected pregnant women [13 20]
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19 319 or HIV-infected adults [21 22] in Tanzania. The major difference is that in our earlier
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21 320 studies [13 20], a large majority (~85%) of the participants had stage 1 or asymptomatic
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23 321 HIV disease, whereas in this study, most of the individuals were already at stage 3
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25 322 disease.
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33 324 The association of low vitamin D status with TB recurrence/relapse, primarily driven by
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35 325 the HIV-uninfected subset, is a novel finding in a longitudinal study and has important
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37 326 implications. Vitamin D deficiency has been linked to TB in several studies – a
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39 327 hypothesis perhaps initially generated by the observed seasonality of TB. *In vitro* and
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41 328 animal studies indicate that 1,25-dihydroxyvitamin D₃, the most active form of vitamin
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43 329 D, may increase mycobacterial killing by macrophages but also limits host damage by
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45 330 decreasing the gamma-interferon production [23-28]. In perhaps the strongest evidence to
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47 331 date for a role of vitamin D in tuberculosis, a study by Liu *et al* [29] found that the
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49 332 antimycobacterial response in humans is dependent on adequate availability of vitamin D.
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3 334 A few randomized trials of vitamin D supplementation in TB patients have been
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5 335 conducted in the past few years [4 5 30]. In a randomized trial that was conducted among
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7 336 365 TB patients in Guinea-Bissau starting antituberculosis treatment, overall mortality
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9 337 was 15% (54 of 365) at 1 year of follow-up and similar in both arms [5]. Martineau and
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11 338 colleagues didn't find a difference in median time to sputum culture conversion with
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13 339 vitamin D supplementation of 2.5 mg vitamin D3 at enrollment, 14, 28, and 42 days after
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15 340 starting TB treatment in 126 adults with sputum smear-positive PTB [4]. A recent report
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17 341 by Coussens *et al* from a subset of the 126 adults included in the trial above stated that
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19 342 median time to sputum smear conversion in the intervention arm was significantly shorter
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21 343 than in the control arm (23 vs. 36 days; $p=0.04$) [30]. The lack of effect and concordance
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23 344 in most of these trials is probably due to the dose and dosing interval used. It is worth
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25 345 noting however, that large intermittent doses of vitamin D may result in
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27 346 supraphysiological concentrations in some cases, which may be more harmful than
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29 347 helpful in their effects on the immune system [31].
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41 349 Low vitamin D status also was associated with T-cell subset counts only among the HIV-
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43 350 infected patients in this cohort. We can only speculate as to the reasons for the
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45 351 significantly higher increase in CD4 T-cells observed in patients with low vitamin D
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47 352 levels at baseline. One potential explanation is that HIV-infected patients with low
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49 353 vitamin D status may experience more uncontrolled immune reconstitution, leading to a
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51 354 greater increase in CD4 T-cell counts, on treatment of TB, compared to patients with
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53 355 adequate vitamin D status. This may also explain why this relationship is observed only
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55 356 in the first eight months of follow-up and not subsequently.
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7 358 The results for CD8 and CD3 T-cells are consistent with our previous studies among
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9 359 HIV-infected women in Tanzania [13 14]. This could suggest a possible role of vitamin
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11 360 D in inflammation. Although, the conventional role of CD8 cells is as cytotoxic cells,
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13 361 they also are effector cells in inflammation [32]. The involvement of vitamin D in
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15 362 modulating CD8 cells is also indicated by the fact that CD8 cells express the highest
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17 363 concentration of vitamin D receptor of the immune cells [33]. Other studies also have
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19 364 found that vitamin D suppresses antigen-stimulated proinflammatory cytokine responses,
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21 365 which may help speed up resolution of inflammatory responses that can lead to increased
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23 366 risk of mortality among TB patients [30].
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30 368 TB, once known as ‘consumption’, is associated with significant wasting and weight loss.
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32 369 The observation that better vitamin D status among HIV-uninfected patients is associated
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34 370 with a greater increase in BMI during follow-up is likely related to decreased risk of
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36 371 relapse among these patients, as well as improvement in quality of life through
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38 372 mechanisms such as better metabolism that were not directly assessed in this study.
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42 373 The major strengths of this study include a large number of participants, more than half
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44 374 of whom were HIV-infected, comprehensive assessment of clinical, immunological,
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46 375 socio-demographic, and nutritional parameters, and a long duration of follow-up. On the
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48 376 other hand, the major limitation is the possibility of reverse causation and residual
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50 377 confounding. We have attempted to minimize this through rigorous analyses and
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52 378 adjusting for several potential confounders, including hemoglobin concentrations, HIV
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54 379 status, viral load, CD4 T-cells, and Karnofsky score, in most analyses. The study results
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3 380 are generalizable to most settings with a high TB burden and widely prevalent vitamin D
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5 381 insufficiency.
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10 383 In summary, our study results indicate that adequate vitamin D status is associated with
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12 384 better clinical and nutritional parameters during follow-up in a cohort of TB patients in
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14 385 Tanzania. While randomized trials of vitamin D supplementation among TB patients are
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16 386 urgently warranted, it is also imperative to conduct dose-response studies to determine
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18 387 ideal dose and duration for the supplement.
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28 401 The authors report NO conflict of interest
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33 403 *Author Contributions*

34
35 404 SM wrote the first draft of the manuscript and analyzed and interpreted the data; FMM,
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37 405 RJB, SA, WU, EV, and WWF were investigators of the parent trial and contributed to
38
39 406 field activities and oversight; RJB also helped with the analysis and interpretation of the
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41 407 data; all authors participated in study design and contributed to the final manuscript. All
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43 408 authors have also read and approved the final manuscript.
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References

1. WHO. Global Tuberculosis Control. Geneva: World Health Organization, 2011.
2. Chaisson RE, Martinson NA. Tuberculosis in Africa--combating an HIV-driven crisis. *N Engl J Med* 2008;**358**(11):1089-92 doi: 358/11/1089 [pii] 10.1056/NEJMp0800809[published Online First: Epub Date]].
3. Martineau AR, Nhamoyebonde S, Oni T, et al. Reciprocal seasonal variation in vitamin D status and tuberculosis notifications in Cape Town, South Africa. *Proc Natl Acad Sci U S A* 2011;**108**(47):19013-7 doi: 10.1073/pnas.1111825108[published Online First: Epub Date]].
4. Martineau AR, Timms PM, Bothamley GH, et al. High-dose vitamin D(3) during intensive-phase antimicrobial treatment of pulmonary tuberculosis: a double-blind randomised controlled trial. *Lancet* 2011;**377**(9761):242-50 doi: 10.1016/S0140-6736(10)61889-2[published Online First: Epub Date]].
5. Wejse C, Gomes VF, Rabna P, et al. Vitamin D as supplementary treatment for tuberculosis: a double-blind, randomized, placebo-controlled trial. *American Journal of Respiratory and Critical Care Medicine* 2009;**179**(9):843-50 doi: 10.1164/rccm.200804-567OC[published Online First: Epub Date]].
6. Villamor E, Mugusi F, Urassa W, et al. A Trial of the Effect of Micronutrient Supplementation on Treatment Outcome, T Cell Counts, Morbidity, and Mortality in Adults with Pulmonary Tuberculosis. *J Infect Dis* 2008;**197**(11):1499-505 doi: 10.1086/587846 10.1086/587846 [pii][published Online First: Epub Date]].

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7. Karnofsky DA, Abelmann WH, Craver LF, et al. The use of nitrogen mustards in the palliative treatment of cancer. *Cancer* 1948;**1**:634-56
8. Fawzi WW, Msamanga GI, Spiegelman D, et al. A randomized trial of multivitamin supplements and HIV disease progression and mortality. *N Engl J Med* 2004;**351**(1):23-32
9. Baum M, Cassetti L, Bonvehi P, et al. Inadequate dietary intake and altered nutrition status in early HIV-1 infection. *Nutrition* 1994;**10**(1):16-20
10. Lohman TG, Roche AF, Martorell R. *Anthropometric standardization reference manual*. Champaign, IL: Human Kinetics Books, 1988.
11. WHO. Interim proposal for a WHO staging system for HIV infection and disease. *Wkly Epidemiol Rec* 1990;**65**:221-24
12. Friis H, Range N, Pedersen ML, et al. Hypovitaminosis D is common among pulmonary tuberculosis patients in Tanzania but is not explained by the acute phase response. *J Nutr* 2008;**138**(12):2474-80 doi: 10.1093/ajph/98.12.2474 [pii] 10.3945/jn.108.094979[published Online First: Epub Date].
13. Mehta S, Giovannucci E, Mugusi FM, et al. Vitamin D status of HIV-infected women and its association with HIV disease progression, anemia, and mortality. *PLoS ONE* 2010;**5**(1):e8770 doi: 10.1371/journal.pone.0008770[published Online First: Epub Date].
14. Mehta S, Spiegelman D, Aboud S, et al. Lipid-soluble vitamins A, D, and E in HIV-infected pregnant women in Tanzania. *European journal of clinical nutrition* 2010;**64**(8):808-17 doi: 10.1038/ejcn.2010.76[published Online First: Epub Date].
15. Davies PD, Church HA, Bovornkitti S, et al. Altered vitamin D homeostasis in tuberculosis. *Intern Med (Thailand)* 1988;**4**:45-47

- 1
2
3 16. Norman AW. Nutritional Aspects of Vitamin D. <http://vitamind.ucr.edu/nutri.html>.
4
5 Accessed Oct 9, 2006. 1999. <http://vitamind.ucr.edu/nutri.html> (accessed Oct 9, 2006).
6
7
8 17. Utiger RD. The need for more vitamin D. *N Engl J Med* 1998;**338**(12):828-9
9
10 18. Holick MF. High prevalence of vitamin D inadequacy and implications for health.
11
12 *Mayo Clin Proc* 2006;**81**(3):353-73
13
14 19. Greenland S. Modeling and variable selection in epidemiologic analysis. *Am J Public*
15
16 *Health* 1989;**79**(3):340-9
17
18 20. Mehta S, Mugusi FM, Spiegelman D, et al. Vitamin D status and its association with
19
20 morbidity including wasting and opportunistic illnesses in HIV-infected women in
21
22 Tanzania. *AIDS Patient Care STDS* 2011;**25**(10):579-85 doi:
23
24 10.1089/apc.2011.0182[published Online First: Epub Date].
25
26
27 21. Sudfeld CR, Giovannucci EL, Isanaka S, et al. Vitamin D Status and Incidence of
28
29 Pulmonary Tuberculosis, Opportunistic Infections, and Wasting Among HIV-Infected
30
31 Tanzanian Adults Initiating Antiretroviral Therapy. *The Journal of infectious diseases*
32
33 2013;**207**(3):378-85 doi: 10.1093/infdis/jis693[published Online First: Epub Date].
34
35
36 22. Sudfeld CR, Wang M, Aboud S, et al. Vitamin D and HIV progression among
37
38 Tanzanian adults initiating antiretroviral therapy. *PloS one* 2012;**7**(6):e40036 doi:
39
40 10.1371/journal.pone.0040036[published Online First: Epub Date].
41
42
43 23. Thoma-Uszynski S, Stenger S, Takeuchi O, et al. Induction of direct antimicrobial
44
45 activity through mammalian toll-like receptors. *Science* 2001;**291**(5508):1544-7
46
47
48 24. Waters WR, Palmer MV, Nonnecke BJ, et al. Mycobacterium bovis infection of
49
50 vitamin D-deficient NOS2^{-/-} mice. *Microb Pathog* 2004;**36**(1):11-7
51
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56
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60
25. Waters WR, Nonnecke BJ, Foote MR, et al. Mycobacterium bovis bacille Calmette-Guerin vaccination of cattle: activation of bovine CD4+ and gamma delta TCR+ cells and modulation by 1,25-dihydroxyvitamin D3. *Tuberculosis (Edinb)* 2003;**83**(5):287-97
26. Waters WR, Nonnecke BJ, Rahner TE, et al. Modulation of Mycobacterium bovis-specific responses of bovine peripheral blood mononuclear cells by 1,25-dihydroxyvitamin D(3). *Clin Diagn Lab Immunol* 2001;**8**(6):1204-12
27. Kawakami K, Teruya K, Tohyama M, et al. [A therapeutic trial of experimental tuberculosis with gamma-interferon in an immunocompromised mouse model]. *Kekkaku* 1994;**69**(10):607-13
28. McMurray DN, Bartow RA, Mintzer CL, et al. Micronutrient status and immune function in tuberculosis. *Ann N Y Acad Sci* 1990;**587**:59-69
29. Liu PT, Stenger S, Li H, et al. Toll-Like Receptor Triggering of a Vitamin D-Mediated Human Antimicrobial Response. *Science* 2006:1123933 doi: 10.1126/science.1123933[published Online First: Epub Date].
30. Coussens AK, Wilkinson RJ, Hanifa Y, et al. Vitamin D accelerates resolution of inflammatory responses during tuberculosis treatment. *Proc Natl Acad Sci* 2012:Epub
31. Martineau AR. Bolus-dose vitamin D and prevention of childhood pneumonia. *Lancet* 2012;**379**(9824):1373-5 doi: 10.1016/S0140-6736(12)60405-X[published Online First: Epub Date].
32. Babbe H, Roers A, Waisman A, et al. Clonal expansions of CD8(+) T cells dominate the T cell infiltrate in active multiple sclerosis lesions as shown by micromanipulation and single cell polymerase chain reaction. *J Exp Med* 2000;**192**(3):393-404

1
2
3 33. Veldman CM, Cantorna MT, DeLuca HF. Expression of 1,25-dihydroxyvitamin D(3)
4 receptor in the immune system. Arch Biochem Biophys 2000;374(2):334-8 doi:
5
6 10.1006/abbi.1999.1605
7
8 S0003-9861(99)91605-3 [pii][published Online First: Epub Date].
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Figure Legends

1. Distribution of Vitamin D concentrations at baseline (nmol/L)
2. Distribution of Vitamin D concentrations by season of blood draw; Season 1: Dry (January-February); Season 2: Long Rains (March-June); Season 3: Dry (July-October); Season 4: Short Rains (November-December)

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Tables

Table 1	Baseline Characteristics of the Study Population (n=677)		
		HIV-infected (n=344)	HIV-uninfected (n=333)
Variable		Mean (Standard Deviation)	Mean (Standard Deviation)
Age, years		34.4 ± 8.6	30.2 ± 9.2
Money spent on food per person per day, Tanzanian Shillings*		587.3 ± 445.9	580.1 ± 684.2
Hemoglobin, g/dL		9.9 ± 1.8	11.1 ± 1.7
Albumin, g/dL		2.8 ± 1.0	3.2 ± 1.1
CD3 T-cell count, cells/μL		1228.0 ± 608.5	1195.9 ± 404.8
CD4 T-cell counts, cells/μL		327.2 ± 246.2	709.2 ± 250.8
CD8 T-cell counts, cells/μL		826.9 ± 447.5	427.5 ± 188.2
Log(10) Viral Load, copies/mL		4.6 ± 1.0	N/A
Body Mass Index, kg/m ²		19.4 ± 2.8	18.8 ± 2.5
Mid-Upper Arm Circumference, cm		23.4 ± 2.7	23.1 ± 2.7
Follow-up time, days		916.8 ± 507.4	1532.9 ± 331.4
		n (%)	n (%)
Low Vitamin D (serum 25-hydroxyvitamin D <75 nmol/L)		218 (63.4%)	200 (60.1%)

	Vitamin D deficiency (serum 25-hydroxyvitamin D <50 nmol/L)	55 (16.0%)	51 (15.3%)
	Sex		
	Male	203 (59.0%)	257 (77.2%)
	Female	141 (41.0%)	76 (22.8%)
	Center		
	Mwananyamala	79 (23.0%)	88 (26.4%)
	Temeke	102 (29.7%)	83 (24.9%)
	Tandale	83 (24.1%)	91 (27.3%)
	Mbgala	31 (9.0%)	70 (21.0%)
	Amana	49 (14.2%)	1 (.3%)
	Karnofsky Score <70%	45 (13.1%)	29 (8.7%)
	Education Group		
	None	29 (8.4%)	36 (10.8%)
	Low <5years	35 (10.2%)	31 (9.3%)
	Primary 5-8 years	238 (69.2%)	233 (70.0%)
	Secondary/University	42 (12.2%)	33 (9.9%)

	Cohabits with a Partner	200 (58.1%)	168 (50.5%)
	Assets at home		
	None	92 (26.9%)	108 (32.4%)
	One	89 (26.0%)	85 (25.5%)
	2-3	122 (35.7%)	114 (34.2%)
	4-5	39 (11.4%)	26 (7.8%)
	WHO HIV Disease Stage		
	3	240 (90.9%)	N/A
	4	24 (9.1%)	
	CD4 T-cell categories, cells/ μ L		
	0-199	97 (35.9%)	0 (.0%)
	200-499	116 (43.0%)	69 (22.9%)
	500+	57 (21.1%)	232 (77.1%)
	WHO BMI Group, kg/m ²		
	<16	26 (7.7%)	33 (9.9%)
	16-16.99	37 (10.9%)	45 (13.6%)
	17-18.49	73 (21.5%)	88 (26.5%)
	18.5-19.99	79 (23.3%)	70 (21.1%)
	20-21.99	77 (22.7%)	69 (20.8%)

	22+	47 (13.9%)	27 (8.1%)
* 1 US Dollar \cong 1000 Tanzanian Shillings at the time of the study			

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Table 2		Vitamin D Status and Mortality and HIV Disease Progression in HIV-infected TB Patients			
		Univariate		Multivariate	
Outcome	n/N (%)	RR (95% CI)	p-value	RR (95% CI)	p-value
Mortality					
Low Vitamin D (<75 nmol/L)	61/218 (28.0%)	0.73 (0.50, 1.08)	0.12	0.70 (0.47, 1.04)	0.08
Adequate Vitamin D	43/126 (34.1%)				
Vitamin D deficient (<50 nmol/L)	20/55 (36.4%)	1.34 (0.82, 2.18)	0.25	0.91 (0.55, 1.50)	0.71
Not deficient	84/289 (29.1%)				
Continuous Vitamin D (nmol/L)		1.00 (0.99, 1.01)	0.49	1.01 (1.00, 1.02)	0.15
HIV Disease Progression					
Low Vitamin D	46/150	1.10 (0.67, 1.82)	0.71	1.08 (0.64, 1.82)	0.78

	(<75 nmol/L)	(30.7%)				
	Adequate	23/90		Reference		Reference
	Vitamin D	(25.6%)				
	Vitamin D	14/34		1.91 (1.05, 3.44)	0.03	1.48 (0.78, 2.82)
	deficient (<50	(41.2%)				0.23
	nmol/L)					
	Not deficient	55/206		Reference		Reference
		(26.7%)				
	Continuous Vitamin D			0.99 (0.98, 1.01)	0.30	1.00 (0.99,
	(nmol/L)					1.01)
						0.57
p-values obtained using Cox Proportional Hazards Regression; RR: Risk Ratio; 95%						
CI: 95% Confidence Interval						
Multivariate analyses adjusted for Age, Karnofsky Score, Baseline Hemoglobin, Viral Load,						
HIV Status, CD4 Counts, and Micronutrient Supplementation						

Table 3		Vitamin D Status and Treatment Outcomes in TB Patients				
		Univariate			Multivariate	
Outcome	n/N (%)	RR (95% CI)	p-value	RR (95% CI)	p-value	
Treatment Failure by 1 month post-treatment initiation						
Low Vitamin D (<75 nmol/L)	58/298 (19.5%)	1.06 (0.72, 1.55)	0.77	1.02 (0.70, 1.49)	0.93	
Adequate Vitamin D	34/185 (18.4%)					
Vitamin D deficient (<50 nmol/L)	15/75 (20.0%)	1.06 (0.65, 1.74)	0.82	1.13 (0.69, 1.86)	0.63	
Not deficient	77/408 (18.9%)					
Continuous Vitamin D (nmol/L)		1.00 (0.99, 1.01)	0.49	1.00 (0.99, 1.01)	0.50	
Any Relapse (relapse after 1 month post-treatment initiation if culture negative at 1 month)						
Low Vitamin D (<75 nmol/L)	51/227 (22.5%)	1.56 (0.98, 2.48)	0.06	1.66 (1.04, 2.64)	0.03	

	nmol/L)						
	Adequate Vitamin D	21/146 (14.4%)					
	Vitamin D deficient (<50 nmol/L)	13/56 (23.2%)	1.25 (0.73, 2.12)	0.41	1.40 (0.82, 2.39)	0.21	
	Not deficient	59/317 (18.6%)					
	Continuous Vitamin D (nmol/L)		0.99 (0.98, 1.00)	0.06	0.99 (0.98, 1.00)	0.04	
p-values obtained using Binomial Regression; RR: Risk Ratio; 95% CI: 95% Confidence Interval							
Multivariate analyses adjusted for Age, Karnofsky Score, Baseline Hemoglobin, Viral Load, HIV Status, CD4 Counts, and Micronutrient Supplementation							

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Table 4	Vitamin D Status and T-cell Counts (cells/ μ L) in TB Patients													
	CD4 T-cells					CD8 T-cells					CD3 T-cells			
Outcome		Adequate vitamin D, mean difference (95% CI) ^a	Low vitamin D, adjusted mean difference (95% CI) ^c		p-value	Adequate vitamin D, mean difference (95% CI) ^a	Low vitamin D, adjusted mean difference (95% CI) ^b	Low vitamin D, adjusted mean difference (95% CI) ^c		p-value	Adequate vitamin D, mean difference (SD) ^a	Low vitamin D, mean difference (95% CI) ^b	Low vitamin D, adjusted mean difference (95% CI) ^c	p-value
Entire follow-up: HIV-infected patients														

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Low Vitamin D (<75 nmol/L)	300 (234)	17 (-23, 56)	21 (-18, 59)	0.2 9	902 (457)	88 (7, 169)	85 (4, 165)	0.0 4	1298 (635)	101 (-4, 206)	103 (-5, 212)	0.0 6
Vitamin D deficient (<50 nmol/L)	333 (225)	21 (-34, 76)	30 (-26, 86)	0.2 9	957 (424)	105 (-9, 219)	114 (-6, 234)	0.0 6	1392 (595)	104 (-47, 255)	125 (-28, 279)	0.1 1
Continuous Vitamin D (per nmol/L)		0 (-1, 1)	-1 (-1, 0)	0.2 6		-3 (-5, -1)	-3 (-5, -1)	0.0 04		-3 (-5, -1)	-3 (-6, -1)	0.0 1
Entire follow-up: HIV-uninfected patients												
Low Vitamin D (<75 nmol/L)	771 (235)	-2 (-49, 49)	3 (-45, 51)	0.9 1	508 (209)	-25 (-63, 14)	-22 (-60, 17)	0.2 7	1351 (400)	-37 (-109, 109)	-28 (-99, 44)	0.4 5

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	Vitamin D deficient (<50 nmol/L)	781 (241)	-34 (-99, 30)	-34 (-101, 32)	0.3 1	500 (195)	-1 (-64, 62)	3 (-61, 67)	0.9 3	1354 (397)	-33 (-136, 71)	-28 (-134, 79)	0.6 1
	Continuous Vitamin D (per nmol/L)		0 (-1, 1)	0 (-1, 1)	0.9 7		0 (-1, 1)	0 (-1, 1)	0.8 3		0 (-1, 2)	0 (-2, 2)	0.9 0
First 8 months of follow-up: HIV-infected patients													
	Low Vitamin D (<75 nmol/L)	316 (237)	54 (8, 100)	58 (13, 104)	0.0 1	868 (470)	132 (29, 235)	119 (15, 223)	0.0 2	1279 (670)	190 (42, 337)	179 (28, 331)	0.0 2

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Vitamin D deficient (<50 nmol/L)	372 (264)	36 (-25, 97)	41 (-20, 101)	0.19	963 (471)	63 (-77, 203)	75 (-72, 221)	0.32	1443 (689)	67 (-125, 259)	101 (-93, 295)	0.31
Continuous Vitamin D (per nmol/L)		-1 (-2, 0)	-1 (-2, 0)	0.01		-4 (-6, -1)	-4 (-6, -1)	0.02		-4 (-7, -1)	-5 (-8, -2)	0.03
First 8 months of follow-up: HIV-uninfected patients												
Low Vitamin D (<75 nmol/L)	724 (243)	1 (-52, 53)	6 (-47, 59)	0.82	461 (232)	-22 (-63, 20)	-17 (-57, 22)	0.39	1248 (446)	-38 (-121, 46)	-27 (-106, 52)	0.50
Vitamin D deficient (<50 nmol/L)	731 (237)	-7 (-95, 96)	-7 (-81, 81)	0.87	454 (209)	4 (-73, 81)	5 (-71, 81)	0.90	1247 (410)	14 (-124, 123)	17 (-123, 123)	0.82

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	nmol/L))	80))							153)	156)	
	Continuous Vitamin D (per nmol/L)		0 (- 2,1)	0 (- 2,1)	0.5 4		0 (-1, 1)	0 (-1, 1)	0.7 3		0 (-2, 1)	0 (-2, 1)	0.5 7

^aData are the means (SD) of the average measurement during follow-up for each participant

^bData are the mean difference between the low and the adequate vitamin D group, as defined in Column B. The mean differences, 95% confidence intervals (CIs), and corresponding p-values were estimated from generalized estimating equations, after adjustment for baseline measurements, follow-up time, and treatment (micronutrients vs. placebo) group.

^cMultivariate analyses additionally adjusted for Age, Karnofsky Score, and Baseline Hemoglobin

Table 5 Vitamin D Status and Nutritional Parameters in TB Patients												
Outcome	Body Mass Index (kg/m ²)				Albumin concentration (g/dL)				Hemoglobin concentration (g/dL)			
	Adequate vitamin D, mean (SD) ^a	Low vitamin D, mean difference (95% CI) ^b	Low vitamin D, adjusted mean difference (95% CI) ^c	p-value	Adequate vitamin D, mean (SD) ^a	Low vitamin D, mean difference (95% CI) ^b	Low vitamin D, adjusted mean difference (95% CI) ^c	p-value	Adequate vitamin D, mean (SD) ^a	Low vitamin D, mean difference (95% CI) ^b	Low vitamin D, adjusted mean difference (95% CI) ^c	p-value
Entire follow-up:												

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All patients													
Low Vitamin D (<75 nmol/L)	21.20 (2.80)	-0.06 (-0.30, 0.17)	-0.08 (-0.30, 0.14)	0.46	3.42 (0.74)	-0.05 (-0.14, 0.04)	0.00 (-0.08, 0.08)	0.9 7	12.65 (1.80)	-0.16 (-0.40, 0.08)	-0.18 (-0.41, 0.05)	0. 12	
Vitamin D deficient (<50 nmol/L)	21.23 (3.00)	-0.16 (-0.46, 0.14)	-0.14 (-0.44, 0.15)	0.34	3.42 (0.72)	-0.05 (-0.17, 0.07)	0.02 (-0.08, 0.13)	0.65	12.42 (1.87)	0.15 (-0.16, 0.45)	0.17 (-0.11, 0.45)	0.2 4	
Continuous Vitamin D (per nmol/L)		0.00 (0.00, 0.01)	0.00 (0.00,	0.30		0.002 (0.00,	0.000 (-	0.90		0.00 (-0.01,	0.00 (0.00,	0.8 5	

			0.01)				0.004)	0.002,			0.01)	0.01)	
								0.002)					
First 8 months of follow-up: All patients													
Low Vitamin D (<75 nmol/L)	20.96 (2.73)	-0.20 (-0.40, -0.01)	-0.21 (-0.39, -0.02)	0.03		3.42 (1.09)	-0.01 (-0.18, 0.16)	0.04 (-0.13, 0.21)	0.65	12.12 (1.85)	-0.01 (-0.28, 0.26)	-0.04 (-0.31, 0.23)	0.7 8
Vitamin D	20.85 (2.84)	0.00 (-0.25, 0.25)	0.04 (-0.21,	0.78		3.41 (1.08)	-0.11 (-0.32,	-0.05 (-0.27,	0.64	11.92 (1.99)	0.16 (-0.18,	0.21 (-0.10,	0.1 9

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deficient (<50 nmol/L)			0.29)				0.10)	0.17)				0.50)	0.53)	
Continuous Vitamin D (per nmol/L)	0.00	0.00	0.38			0.000	0.000	0.87			0.00	0.00	0.5	
	(0.00, 0.01)	(0.00, 0.01)				(- 0.003, 0.005)	(- 0.004, 0.004)				(-0.01, 0.00)	(-0.01, 0.00)	7	

^aData are the means (SD) of the average measurement during follow-up for each participant

^bData are the mean difference between the low and the adequate vitamin D group, as defined in Column B. The mean differences, 95% confidence intervals (CIs), and corresponding p-values were estimated from generalized estimating equations, after adjustment for baseline measurements, follow-up time, and treatment (micronutrients vs. placebo) group.

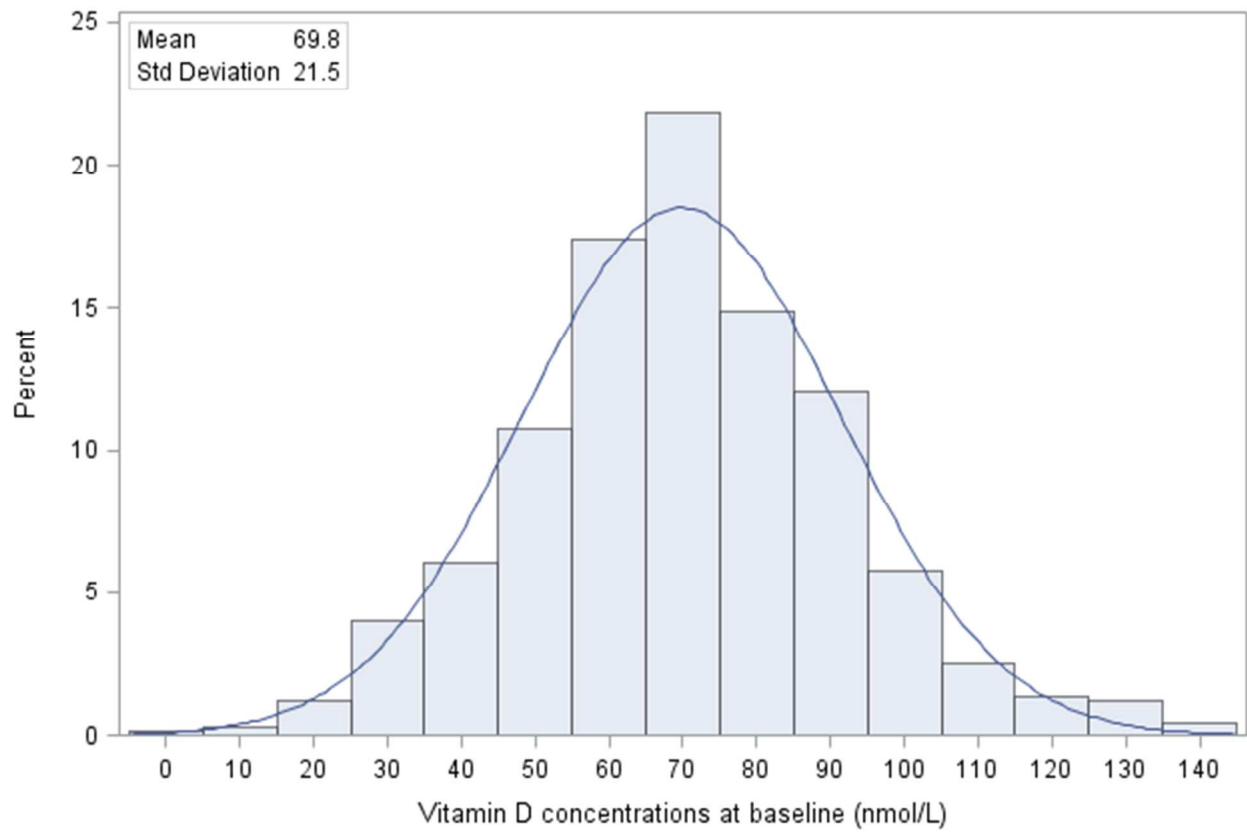
^cMultivariate analyses additionally adjusted for Age, Karnofsky Score, Baseline Hemoglobin, Viral Load, CD4 Count, and HIV Status; HIV status removed from the model where the results are stratified by HIV status. Viral Load also removed from the model in HIV-

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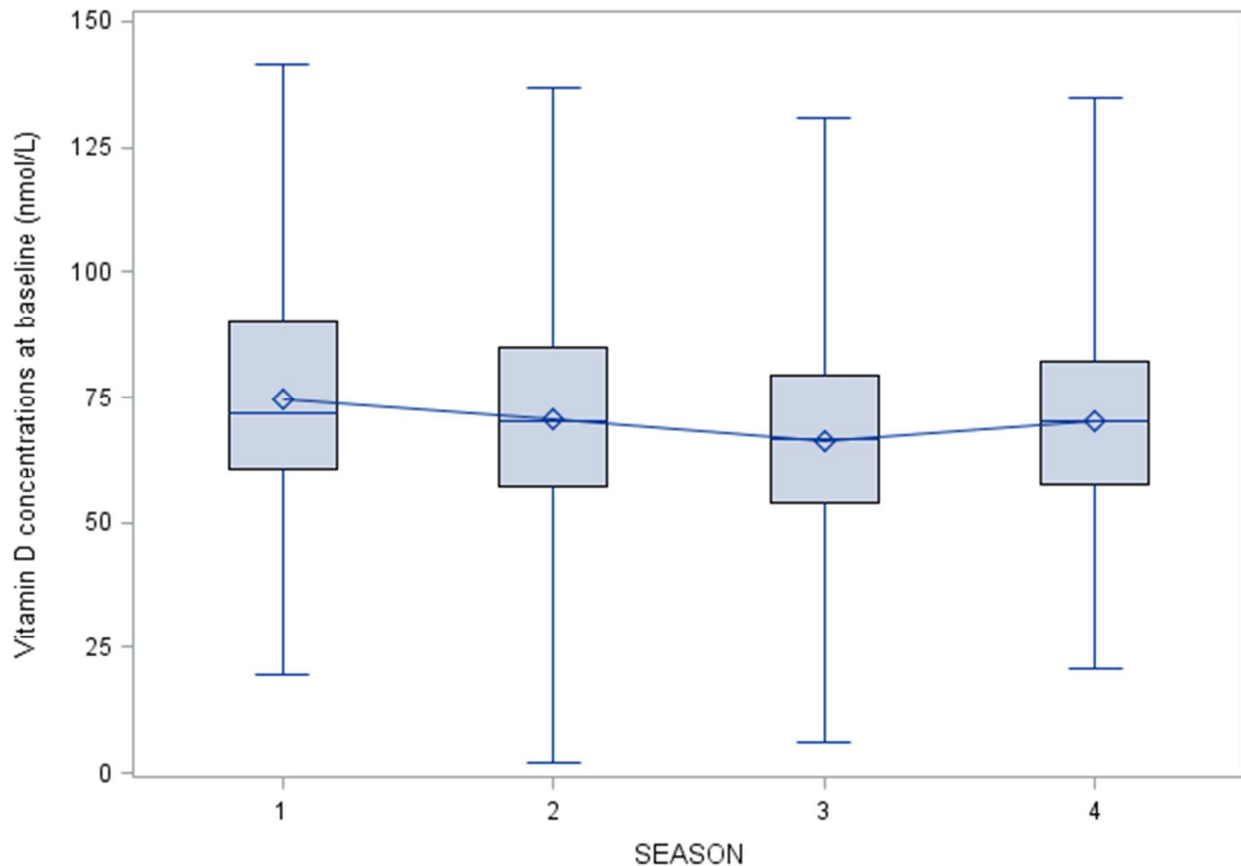
uninfected individuals.

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Supplemental Table 1		Correlates of Low Vitamin D Status (25(OH)D < 75 nmol/L) in HIV-uninfected TB patients			
		Univariate (p<0.20)		Multivariate (p<0.05)	
<i>Variable</i>		RR (95% CI)	p-value	RR (95% CI)	p-value
Season			0.002		0.002
	1: Dry (Jan-Feb)	Ref.		Ref.	
	2: Long Rains (Mar-Jun)	1.07 (0.74, 1.53)		1.09 (0.76, 1.56)	
	3: Dry (Jul-Oct)	1.50 (1.07, 2.09)		1.50 (1.08, 2.08)	
	4: Short Rains (Nov-Dec)	1.29 (0.84, 1.96)		1.26 (0.84, 1.90)	
Sex					
	Female	1.19 (0.99, 1.43)	0.07		
Cohabits with a partner		0.89 (0.75, 1.06)	0.19		
Money spent per person per day on food, per 1000 Tanzanian Shillings*		0.76 (0.59, 0.98)	0.04	0.76 (0.59, 0.98)	0.03
Money spent per person per day on food quartiles			0.14		
	0:<250	1.36 (1.03, 1.80)			
	1:250-499	1.11 (0.82, 1.52)			
	2:500-750	1.13 (0.84, 1.53)			
	3:>750	Ref.			
AFB Culture positive at baseline		1.17 (0.94, 1.46)	0.16		
Number of colonies in AFB culture			0.16		
	1	Ref.			
	2	1.01 (0.66, 1.56)			
	3	0.95 (0.63, 1.44)			
	4	1.05 (0.70, 1.58)			

	5	1.27 (1.01, 1.59)			
Received TB treatment in the past 5 years		1.41 (0.97, 2.04)	0.07		
Hemoglobin, g/dL		0.92 (0.87, 0.97)	0.004		
CD4 T-cells, 100 cells/ μ L		1.03 (0.99, 1.07)	0.12		
CD3 T-cells, 100 cells/ μ L		1.02 (1.00, 1.04)	0.12		
Depressed >2 weeks, ever		1.17 (0.97, 1.42)	0.10		
Dysentery		0.23 (0.04, 1.41)	0.11		
Outpatient visit		1.14 (0.95, 1.38)	0.16		
Skin rash		0.73 (0.46, 1.16)	0.18		
Height quartiles, cm			0.01		0.01
	<158.1	Ref.		Ref.	
	158.1-164.0	1.08 (0.87, 1.32)		1.12 (0.93, 1.36)	
	164.1-169.5	0.76 (0.59, 0.98)		0.82 (0.64, 1.04)	
	169.6+	0.77 (0.60, 0.99)		0.81 (0.64, 1.03)	
Weight, kg		0.99 (0.97, 1.00)	0.03		
WHO BMI groups, kg/m ²			0.14		
	<16	1.14 (0.88, 1.48)			
	16-16.99	0.98 (0.74, 1.29)			
	17-18.49	Ref.			
	18.5-19.99	0.76 (0.57, 1.02)			
	20-21.99	0.89 (0.68, 1.15)			
	22+	1.11 (0.83, 1.48)			
Mid-Upper Arm Circumference (MUAC) <22 cm		1.27 (1.07, 1.51)	0.01		
Triceps Skinfold Thickness,		1.02 (1.00, 1.03)	0.09		

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cm						
p-values obtained using Binomial Regression; RR: Risk Ratio; 95% CI: 95% Confidence Interval; * 1 US Dollar \cong 1000 Tanzanian Shillings at the time of the study						

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Supplemental Table 2		Correlates of Low Vitamin D Status (serum 25(OH)D <75 nmol/L) in HIV-infected TB patients			
		Univariate (p<0.20)		Multivariate (p<0.05)	
Variable		RR (95% CI)	P-value	RR (95% CI)	P-value
Age		0.99 (0.98, 1.00)	0.01		
Center			0.03		
	Mwananyamala	1.05 (0.86, 1.27)			
	Temeke	Ref.			
	Tandale	0.80 (0.63, 1.02)			
	Mbgala	0.67 (0.44, 1.01)			
	Amana	1.03 (0.82, 1.29)			
Cohabits with a partner		0.90 (0.77, 1.05)	0.19		
Assets at home			0.07		
	0:none	1.36 (1.03, 1.80)			
	1:one	0.88 (0.66, 1.16)			
	2:2-3	0.87 (0.67, 1.14)			
	3:4-5	Ref.			
Received TB treatment in the past 5 years		0.58 (0.31, 1.10)	0.10		
Hemoglobin, g/dL		0.95 (0.90, 1.00)	0.04	0.93 (0.89, 0.98)	0.007
Albumin, U/L		0.93 (0.86, 1.02)	0.13		
CD4 T-cells, 100 cells/ μ L		1.02 (0.99, 1.05)	0.12	1.04 (1.01, 1.07)	0.02
Depressed >2 weeks, ever		0.80 (0.61, 1.05)	0.11		
Hospitalization		1.30 (0.94, 1.80)	0.11		
Skin rash		1.32 (1.07, 1.64)	0.01		
Extrapulmonary TB		1.35 (0.93, 1.96)	0.11		

Height, cm		0.99 (0.98, 1.00)	0.08		
Weight, kg		0.99 (0.98, 1.00)	0.11		
WHO Body Mass Index (BMI) groups, kg/m ²			0.06		
	<16	0.81 (0.57, 1.16)			
	16-16.99	0.99 (0.76, 1.27)			
	17-18.49	Ref.			
	18.5-19.99	0.80 (0.63, 1.02)			
	20-21.99	0.75 (0.58, 0.96)			
	22+	1.05 (0.84, 1.30)			
Mid-Upper Arm Circumference (MUAC) quartiles, cm			0.02		
	<=21	Ref.			
	21.1-23.0	1.09 (0.90, 1.32)			
	23.1-24.9	0.81 (0.61, 1.08)			
	25+	0.82 (0.66, 1.03)			
p-values obtained using Binomial Regression; RR: Risk Ratio; 95% CI: 95% Confidence Interval					

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	2
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	2-3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5
Objectives	3	State specific objectives, including any prespecified hypotheses	6
Methods			
Study design	4	Present key elements of study design early in the paper	6-8
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6-8
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	6-8
		(b) For matched studies, give matching criteria and number of exposed and unexposed	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6, 9
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	9-10
Bias	9	Describe any efforts to address potential sources of bias	10
Study size	10	Explain how the study size was arrived at	10
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	10
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	9-10
		(b) Describe any methods used to examine subgroups and interactions	9-10
		(c) Explain how missing data were addressed	9-10
		(d) If applicable, explain how loss to follow-up was addressed	9-10
		(e) Describe any sensitivity analyses	NA
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	10, Tables
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	10-11
		(b) Indicate number of participants with missing data for each variable of interest	10
		(c) Summarise follow-up time (eg, average and total amount)	11
Outcome data	15*	Report numbers of outcome events or summary measures over time	Tables
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Tables
		(b) Report category boundaries when continuous variables were categorized	Tables
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	10-13
Discussion			
Key results	18	Summarise key results with reference to study objectives	13
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	14-19
Generalisability	21	Discuss the generalisability (external validity) of the study results	18-19
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	20

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.



Vitamin D Status and TB Treatment Outcomes in Adult Tanzanian Patients

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Manuscripts

Vitamin D Status and TB Treatment Outcomes in Adult Tanzanian Patients

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5 2 **Objectives:** Vitamin D is an immunomodulator and can alter response to tuberculosis
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8 3 treatment, though randomized trials have been inconclusive to date. We present the first
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10 4 comprehensive analysis of the associations between vitamin D status and TB treatment,
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13 5 T-cell counts, and nutritional outcomes by HIV status.
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17 7 **Design:** Cohort study
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22 9 **Setting:** Outpatient clinics in Tanzania
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27 11 **Participants:** 25-hydroxyvitamin D levels were assessed in a cohort of 677 patients with
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29 12 TB (344 HIV-infected) initiating anti-TB treatment at enrollment in a multivitamin
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31 13 supplementation (excluding vitamin D) trial (Clinicaltrials.gov identifier:
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33 14 NCT00197704).
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38 16 **Primary and secondary outcome measures:** Information on treatment outcomes such as
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40 17 failure and relapse, HIV disease progression, T-cell counts, and anthropometry was
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42 18 collected routinely, with a median follow-up of 52 and 30 months for HIV-uninfected and
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44 19 HIV-infected patients, respectively. Cox and binomial regression, and generalized
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46 20 estimating equations were used to assess the association of vitamin D status with these
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48 21 outcomes.
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4 23 **Results:** Mean 25-hydroxyvitamin D concentrations at enrollment were 69.8 (\pm 21.5)
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6 24 nmol/L [27.9 (\pm 8.6) ng/mL]. Vitamin D insufficiency (<75 nmol/L) was associated with
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8 25 a 66% higher risk of relapse (95% CI: 4%, 164%; 133% higher risk in HIV-uninfected
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10 26 patients). Each unit higher vitamin D levels at baseline were associated with a decrease of
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12 27 3 ($p=0.004$) CD8 and 3 ($p=0.01$) CD3 T-cells/ μ L during follow-up in HIV-infected
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14 28 patients. Vitamin D insufficiency was also associated with a greater decrease of BMI (-
15
16 29 0.21 kg/m²; 95% CI:-0.39, -0.02), during the first eight months of follow-up. No
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18 30 association was observed for vitamin D status with mortality or HIV disease progression.
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24 32 **Conclusions:** Adequate vitamin D status is associated with a lower risk of relapse and
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26 33 with improved nutritional indicators such as BMI in TB patients, with or without HIV
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28 34 infection. Further research is needed to determine the optimal dose of vitamin D and
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30 35 effectiveness of daily vitamin D supplementation among patients with TB.
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3 38 **Article Focus:** Recent laboratory data has suggested that optimal vitamin D status may
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5 39 be associated with a more effective immune response to TB infection, a faster rate of
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7 40 bacteriologic cure, and better long-term outcomes. However, clinical and epidemiological
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9 41 studies have found inconsistent results. In this paper, we present the first comprehensive
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11 42 analysis of the associations between vitamin D status and TB treatment, T-cell counts,
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13 43 and nutritional outcomes by HIV status.
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20 45 **Key Messages:** We found that patients with adequate vitamin D status were less likely to
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22 46 experience a relapse during follow-up after completing TB treatment. They were also
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24 47 more likely to have a better nutritional status, as assessed by their body mass index,
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26 48 during follow-up, compared to patients with vitamin D insufficiency. The results provide
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28 49 justification for conducting both a dose response study to determine optimal dose of
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30 50 vitamin D and a randomized controlled trial of daily vitamin D supplementation among
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32 51 patients with TB.
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39 53 **Strengths and Limitations of this study:** The major strengths of this study include a
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41 54 large number of participants, more than half of whom were HIV-infected, comprehensive
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43 55 assessment of clinical, immunological, socio-demographic, and nutritional parameters,
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45 56 and a long duration of follow-up. On the other hand, the major limitation is the possibility
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47 57 of reverse causation and residual confounding. We have attempted to minimize this
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49 58 through rigorous analyses and adjusting for several potential confounders, including
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51 59 hemoglobin concentrations, HIV status, viral load, CD4 T-cells, and Karnofsky score, in
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53 60 most analyses.
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3 61 **Introduction**
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5 62 *Mycobacterium tuberculosis* is one of the most pernicious infectious diseases and
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8 63 successful pathogens known to man. More than 95% of the estimated cases and deaths
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10 64 due to tuberculosis (TB) occur in low-income countries. The United Republic of
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12 65 Tanzania is one of the 22 high-burden countries that account for 80% of global TB cases.
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14 66 Tanzania has an incidence of 177 cases per 100,000 population per year and a prevalence
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16 67 of 183 cases per 100,000 population per year [1].
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22 69 The spread of Human Immunodeficiency Virus (HIV) has fuelled the resurgence of the
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24 70 TB epidemic in Tanzania, as in other parts of sub-Saharan Africa [2]. HIV is the
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26 71 strongest factor in the development of active TB; it is estimated that only one out of ten
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28 72 immunocompetent persons infected with TB develops active TB in his/her lifetime;
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30 73 whereas, one out of ten HIV-infected persons infected with TB will develop active TB
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32 74 every year. An estimated 38% of TB patients in Tanzania are also infected with HIV [1].
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38 76 Current treatment regimens, given under appropriate management conditions, are nearly
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40 77 100% curative for patients with drug-susceptible organisms. However, in Tanzania,
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42 78 treatment fails in 12-17% of the cases. Additionally, TB patients in settings such as
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44 79 Tanzania grapple with multiple health-related and quality of life issues, which are not
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46 80 addressed adequately with treatment alone.
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52 82 Recent data has suggested that optimal vitamin D status may be associated with a more
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54 83 effective immune response to TB infection, a faster rate of bacteriologic cure, and better
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3 84 long-term outcomes. For example, a recent cross-sectional study found that vitamin D
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5 85 deficiency is highly prevalent in South Africa and is associated with susceptibility to
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8 86 active TB both in the presence and absence of HIV infection [3]. A few randomized trials
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11 87 have also been conducted; two of the recent ones failed to find an effect of vitamin D
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13 88 supplementation on treatment success [4 5]. However, the dose used and duration of
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15 89 supplementation may have precluded finding an effect. Further, most studies had small
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17 90 sample sizes and assessed only a limited number of covariates.
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22 92 In this manuscript, we comprehensively examined the hypotheses that vitamin D status
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24 93 may be associated with response to treatment, risk of treatment failure, laboratory
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26 94 parameters such as T-cell counts, and anthropometric measurements in the context of a
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28 95 randomized trial of micronutrient supplementation (supplement did not contain vitamin
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30 96 D) in Tanzania to better inform future studies or trials.
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35 98 ***Materials and Methods***

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37 99 Study Population: The study population and recruitment methods have been described in
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39 100 detail earlier [6]. Briefly, 887 adults with pulmonary tuberculosis (PTB) were enrolled in
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41 101 a randomized trial (Clinicaltrials.gov identifier: NCT00197704) to examine the effects of
42
43 102 micronutrient supplementation on TB treatment failure, relapse, and mortality. The trial
44
45 103 started in April 2000 in Dar es Salaam, Tanzania and continued until April 2005. The
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47 104 eligibility criteria for the study included positive sputum smears for acid-fast bacilli
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49 105 (AFB), age between 18 and 65 years, Karnofsky performance score of $\geq 40\%$ [7], plan to
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51 106 stay in Dar es Salaam for 2 years, not being pregnant, and not having received anti-TB
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3 107 treatment during the previous one year. Consenting subjects were randomly assigned in
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5 108 computer-generated permuted blocks of 20, stratified by HIV status, to receive a daily
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8 109 oral dose of 1 of 2 regimens: micronutrients (5000 IU of retinol, 20 mg of vitamin B1,
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10 110 20mg of vitamin B2, 25mg of vitamin B₆, 100 mg of niacin, 50 µg of vitamin B₁₂, 500
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12 111 mg of vitamin C, 200 mg of vitamin E, 0.8 mg of folic acid, and 100 µg of selenium) or
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14 112 placebo. These doses represent between 6 and 10 times the recommended dietary
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16 113 allowance (RDA) and were being tested at the time among HIV-infected adults from this
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18 114 setting [8]. We chose multiples of the RDA because previous observational studies
19
20 115 suggested that HIV-infected individuals need higher dietary intakes of micronutrients to
21
22 116 achieve normal serum concentrations [9]. All patients received a daily combination of
23
24 117 rifampicin, isoniazid, pyrazinamide, and ethambutol under direct observation of a health
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26 118 worker during the first 2 months (intensive phase) followed by 6 months of self-
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28 119 administered daily isoniazid and ethambutol, as per the Tanzania National TB and
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30 120 Leprosy Programme guidelines. None of the HIV-infected patients received antiretroviral
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32 121 therapy, as these medications were not routinely available in Tanzania at the time this
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34 122 trial was conducted.
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43 124 At the time of randomization, research nurses collected information on various socio-
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45 125 demographic characteristics including age, education levels, marital status, and
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47 126 socioeconomic status. Anthropometric measurements were also obtained using
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49 127 standardized procedures [10] at the randomization visit as well as during each monthly
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51 128 follow-up visit. Height was measured to the nearest 0.1 cm using SECA Bodymeter 206
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53 129 stadiometers, weight to the nearest 100 g with SECA 700 balance beam scales, and left
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3 130 mid-upper arm circumference (MUAC) at the midpoint between the acromion and
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5 131 olecranon to the nearest 0.1 cm using non-stretchable tailor's tapes.
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10 133 Physician visits were scheduled every 3 months. During these visits, study physicians
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12 134 inquired about the health of the subject during the preceding period and performed a
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14 135 complete physical examination. The stage of HIV disease was assessed according to the
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16 136 World Health Organization system [11].
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22 138 Ethics Approval: A written informed consent was obtained from all the study
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24 139 participants. The institutional review boards of the Muhimbili University of Health and
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26 140 Allied Sciences, the Tanzanian National AIDS Control Program, and the Harvard School
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28 141 of Public Health approved the study protocol.
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34 143 Laboratory Methods: At the time of initiation of anti-TB treatment, HIV status was
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36 144 assessed among consenting patients using 2 sequential ELISAs (Wellcozyme, Murex
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38 145 Biotech; Enzygnost anti-HIV1/2 Plus, Dade Behring); discrepant results were resolved by
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40 146 Western Blot test (Bio-rad, Genetic Systems). Both pre-test and post-test counseling was
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42 147 provided. A blood sample also was obtained for measurement of hemoglobin and
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44 148 albumin concentrations using AcT Diff II hematology analyzer (Beckman Coulter,
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46 149 Miami) and Hitachi 911 analyzer (Roche Diagnostics), respectively. CD4, CD3, and CD8
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48 150 T-cell counts were determined using FACScout or FACSCan systems (Becton
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50 151 Dickinson, CA, USA). Viral load was also determined using the Amplicor HIV-1
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52 152 monitor v1.5 assay (Roche Molecular Systems, Branchburg, NJ, USA).
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5 154 Assessment of Vitamin D Status: Serum 25-hydroxyvitamin D concentrations were
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8 155 measured using liquid chromatography-mass spectrometry at the Children's Hospital in
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10 156 Boston only at enrollment before the initiation of micronutrient supplementation. We
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12 157 defined vitamin D insufficiency status as serum 25(OH)D levels of less than 75 nmol/L
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14 158 and adequate otherwise. Vitamin D deficiency was defined as serum 25(OH)D levels of
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16 159 less than 50 nmol/L.
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22 161 Statistical Analysis: We examined the association of vitamin D status with TB treatment
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24 162 outcomes as well as nutritional, immunological, and clinical end points in the entire
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26 163 cohort and separately by HIV status at baseline. TB-related end points included treatment
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28 164 failure, early relapse, and late relapse. Treatment failure by 1 month was defined as
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30 165 positive AFB cultures at 1 month from the initiation of treatment. Relapses were deemed
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32 166 to have occurred in patients with positive cultures, among those who had become culture
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34 167 negative after treatment initiation. Relapses/recurrences included both endogenous
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36 168 reactivation and exogenous reinfection, which could not be distinguished in this study.
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38 169 We calculated the relative risks (RRs) and 95% confidence intervals (CIs) for each of
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40 170 these outcomes by vitamin D status using binomial regression. We used Cox proportional
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42 171 hazards models to assess the association of vitamin D status with mortality in all patients
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44 172 and HIV disease progression from stage 3 to 4 in HIV-infected participants. We defined
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46 173 the end of follow-up as the date when HIV stage was last assessed.
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3 175 We examined the association of vitamin D status with CD4, CD8, and CD3 T-cell counts,
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5 176 viral load (in HIV-infected participants), indicators of nutritional status (body mass index
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8 177 [BMI] and albumin concentrations), and hemoglobin concentrations using generalized
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10 178 estimating equations (GEEs). These models do not require that all patients have the same
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12 179 number of follow-up assessments or that the follow-up measurements be obtained at
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14 180 exactly the same time points. We assumed a standard normal distribution for repeated
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16 181 continuous end points (T cell subsets, log₁₀ viral load, anthropometry, and albumin and
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18 182 hemoglobin concentrations) and estimated average differences during follow-up by
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20 183 vitamin D status. We used an exchangeable correlation structure to account for within-
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22 184 subject correlations and adjusted the models for the follow-up time when the
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24 185 measurements had been obtained and for the baseline values.
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32 187 We analyzed the data for the entire period and for the first 8 months, coinciding with the
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34 188 expected end of TB treatment. Multivariate analyses adjusted for age, Karnofsky score,
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36 189 baseline hemoglobin concentrations, viral load, HIV status, CD4 T-cell counts, and
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38 190 micronutrient supplementation, unless otherwise specified in the results section or the
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40 191 tables. All analyses were performed using SAS software version 9.3 (SAS Institute Inc.,
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42 192 Cary NC).
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48 194 **Results**

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50 195 Baseline 25-hydroxyvitamin D concentrations were available for 677 patients out of the
51
52 196 original cohort of 887. Mean 25-hydroxyvitamin D concentration was 69.8 (±21.5)
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54 197 nmol/L [27.9 (±8.6) ng/mL] and its distribution is shown in Figure 1. The baseline
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3 198 characteristics of these 677 patients by HIV status are presented in Table 1. 36% of the
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5 199 HIV-infected patients had CD4 T-cell counts below 200 cells/ μ L. The mean body mass
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7 200 index (BMI) was 19.1 ± 2.7 kg/m². The median follow-up time for HIV-uninfected
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9 201 patients was 52 months (inter-quartile range [IQR]: 47-57 months) and for HIV-infected
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11 202 patients was 30 months (IQR: 15-41 months).
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18 204 The mean 25-hydroxyvitamin D concentrations were significantly different across season
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20 205 of blood draw in this cohort (p=0.004). Tanzania has four seasons: dry (January-
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22 206 February); long rains (March-June); dry (July-October); short rains (November-
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24 207 December). The boxplot of vitamin D's association with season of blood draw is
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26 208 presented in Figure 2. In subgroup analyses, this association was only observed among
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28 209 the HIV-uninfected patients and not the HIV-infected patients.
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34 211 We examined the correlates of vitamin D insufficiency, defined as serum 25(OH)D
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36 212 concentrations below 75 nmol/L (75 nmol/L) in Supplemental Tables 1 (HIV-uninfected)
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38 213 and 2 (HIV-infected). All factors that had univariate associations with p<0.20 were
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40 214 included in a multivariate model; only the factors that had p<0.05 were retained in the
41
42 215 final model. Among the HIV-uninfected subset, patients enrolled in the dry winter season
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44 216 between July and October were 50% more likely to have vitamin D insufficiency,
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46 217 compared to patients enrolled in the dry summer season between January and February (p
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48 218 for season=0.002). Similarly, the participants with the lowest height were more likely to
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50 219 have vitamin D insufficiency (p=0.01). Finally, greater expenditure on food per person
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52 220 per day was associated with a lower risk of having inadequate vitamin D status (Risk
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3 221 Ratio [RR] per 1000 Tanzanian Shillings [approximately 1 US Dollar at the time of the
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5 222 study]: 0.76; 95% Confidence Interval [CI]: 0.59, 0.98). In the HIV-infected subset,
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7 223 patients with higher hemoglobin concentrations at baseline were less likely to have
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9 224 vitamin D insufficiency, with a 7% lower risk per 1 g/dL higher hemoglobin level
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11 225 ($p=0.007$). On the other hand, higher number of CD4 T-cells was associated with a higher
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13 226 risk of having inadequate vitamin D status (4% higher risk per 100 CD4 T-cells/ μL ;
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15 227 $p=0.02$).

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21 229 There was no significant association of vitamin D status at TB treatment initiation with
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23 230 mortality or HIV disease progression in this cohort (Table 2 includes only HIV-infected
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25 231 participants as there were only 13 deaths in the HIV-uninfected subset). There was no
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27 232 association observed between vitamin D status and treatment failure one month after
28
29 233 initiation of TB treatment (Table 3). However, patients with vitamin D insufficiency (<75
30
31 234 nmol/L) had a 66% higher risk of relapse after becoming culture-negative at one month
32
33 235 after initiation of TB treatment (95% CI: 4%, 164%). This association was more
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35 236 pronounced in those who were not HIV-infected at enrollment in the study (RR: 2.33;
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37 237 95% CI: 1.26, 4.29). In analysis with continuous vitamin D levels, each nmol/L increase
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39 238 was associated with a 1% lower risk of relapse during follow-up ($p=0.04$).

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41 239
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43 240 Vitamin D insufficiency was observed to have no association with CD4 T-cell counts
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45 241 during the entire follow-up in either the HIV-infected or the HIV-uninfected subsets
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47 242 (Table 4). However, vitamin D insufficiency was associated with greater CD4 T-cell
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3 243 counts during the first eight months of follow-up in the HIV-infected patients (mean
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5 244 difference: 58; 95% CI: 13, 104).
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10 246 In analysis among HIV-infected patients with continuous vitamin D levels, each nmol/L
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12 247 higher vitamin D concentration was associated with a decrease of 3 CD8 and 3 CD3 T-
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14 248 cells per μL . Vitamin D insufficiency was associated with an average of 85 higher CD8
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16 249 T-cells/ μL during follow-up (95% CI: 4, 165). Similar results were observed when we
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18 250 restricted the analyses to the first eight months of follow-up, the duration of TB treatment
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20 251 at the time of the study in Tanzania. No relationship was observed with mean viral loads
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22 252 during follow-up in the patients who were HIV-infected at the time of enrollment.
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29 254 In analysis examining association of vitamin D status with nutritional parameters in the
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31 255 entire period of follow-up, no significant relationship was observed with BMI, albumin,
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33 256 or hemoglobin concentrations (Table 5). During the first eight months of follow-up,
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35 257 patients with Vitamin D Insufficiency experienced a decline in BMI (Mean: -0.21 kg/m^2 ;
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37 258 95% CI: $-0.39, -0.02$), compared to patients with adequate vitamin D status. These results
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39 259 were more pronounced in HIV-uninfected patients (Mean: -0.34 ; 95% CI: $-0.60, -0.09$)
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41 260 and not significant in HIV-infected patients. HIV-infected patients with vitamin D
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43 261 insufficiency had increased albumin levels (Mean: 0.94 ; 95% CI: $0.55, 1.32$) during the
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45 262 first eight months of follow-up compared to patients with adequate vitamin D status.
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53 264 ***Discussion***
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3 265 In this study among 677 patients with tuberculosis in Tanzania, more than 61% of the
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5 266 participants had 25-hydroxyvitamin D concentrations below 75 nmol/L (75 nmol/L). 25-
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8 267 hydroxyvitamin D concentrations were associated with the season of blood draw, money
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10 268 spent on food per person per day, and height in HIV-uninfected participants and
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12 269 hemoglobin concentrations and CD4 T-cell counts among HIV-infected patients. Vitamin
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14 270 D insufficiency (<75 nmol/L) was not associated with mortality, HIV disease
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17 271 progression, or treatment failure during follow-up in the entire cohort. However, patients
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19 272 with vitamin D insufficiency had an increased risk of experiencing TB relapse during
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21 273 follow-up. Further, vitamin D insufficiency was associated with a decline in CD8 and
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23 274 CD3 T-cells in both the first eight months (the duration of TB treatment) and the entire
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25 275 period of follow-up. A similar relationship was observed with BMI in the first eight
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27 276 months of follow-up.
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34 278 Our study was conducted in Dar es Salaam, the largest urban center in Tanzania, and just
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36 279 six degrees south of the Equator. The prevalence of vitamin D insufficiency (>61%) in
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38 280 this study is higher than the approximately 40% found in a previous study among TB
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40 281 patients in Mwanza, Tanzania [12] and in our studies among HIV-infected pregnant
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42 282 women (~85% of them had stage 1 HIV disease, unlike this study) in Dar es Salaam [13
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44 283 14]. However, this prevalence is lower than what was observed in a cross-sectional study
45
46 284 in South Africa, where 88% of HIV-uninfected and 97% of HIV-infected TB patients had
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48 285 vitamin D insufficiency. The mean vitamin D concentration in this study was 69.8
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50 286 nmol/L, compared to 86.5 nmol/L in the study in Mwanza and 28.8-40 nmol/L in the
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52 287 South African study. One study from Thailand observed similar levels (69.0 nmol/L) in
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3 288 TB patients [15]; Thailand is located at a similar distance from the Equator as Tanzania,
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5 289 though it is in the northern hemisphere. Similar to the study in South Africa, the vitamin
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8 290 D levels were lowest in our study in the dry winter season between July and October,
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10 291 though the differences were not as stark. For example, the mean vitamin D concentration
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12 292 in January to March in the South African study was 56.8 nmol/L and 30.8 nmol/L
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14 293 between July and September, whereas in our study, the concentrations were 74.8 nmol/L
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16 294 for January through February, and 66.3 nmol/L for July through October.
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22 296 Vitamin D is synthesized in the skin through the action of ultraviolet light on 7-
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24 297 dehydrocholesterol. Fatty fish, such as salmon and sardines, are good sources of vitamin
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27 298 D in the diet but are not widely available everywhere and are usually expensive.
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29 299 Increasing urbanization and a tendency to spend most time indoors are major factors that
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31 300 contribute to the inability of the skin to synthesize adequate amounts of vitamin D [16-
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33 301 18]. Additionally, the TB disease itself and/or the HIV co-infection in the participants in
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35 302 this study are probably the primary reasons for restricted physical activity, lack of
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37 303 adequate exposure to sunlight, and consequent low concentrations of vitamin D.
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43 305 Several other investigators have examined correlates of vitamin D status in TB patients.
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45 306 The study in Mwanza found that marital status, BMI, and serum transferrin receptor
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47 307 concentrations were correlated with vitamin D status. Though the first two were
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49 308 correlated with vitamin D status in our study in univariate analyses, neither remained
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51 309 significant in multivariate analyses. We didn't measure serum transferrin receptor in our
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53 310 study, though we did observe a correlation of vitamin D status with hemoglobin
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3 311 concentrations among the HIV-infected subset. Another study in South Africa found that
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5 312 TB status (active disease vs. latent infection), month of sampling, and BMI were
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7 313 significantly correlated with vitamin D status in multivariate analyses [3]. All patients in
8
9 314 our study had active disease, and we didn't observe a relationship with BMI in our
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11 315 analyses. The study in South Africa incorporated only those correlates associated with
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13 316 serum 25(OH)D concentration with $P < 0.05$ in univariate analysis in the multivariate
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15 317 model. This may have precluded selection of important covariates and confounders, if
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17 318 measured, and produced biased estimates and confidence intervals; increasing the
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19 319 nominal significance level to 20% or more, as used in this study [19] can eliminate most
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21 320 of this bias. Most other studies have been with smaller sample sizes and have examined a
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23 321 limited set of covariates, compared to the current study.
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33 323 There was no association of vitamin D status with mortality or HIV disease progression
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35 324 in this cohort, unlike our previous studies among HIV-infected pregnant women [13 20]
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37 325 or HIV-infected adults [21 22] in Tanzania. The major difference is that in our earlier
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39 326 studies [13 20], a large majority (~85%) of the participants had stage 1 or asymptomatic
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41 327 HIV disease, whereas in this study, most of the individuals were already at stage 3
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43 328 disease.
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49 330 The association of vitamin D insufficiency with TB recurrence/relapse, primarily driven
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51 331 by the HIV-uninfected subset, is a novel finding in a longitudinal study and has important
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53 332 implications. Vitamin D deficiency has been linked to TB in several studies – a
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55 333 hypothesis perhaps initially generated by the observed seasonality of TB. *In vitro* and
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3 334 animal studies indicate that 1,25-dihydroxyvitamin D₃, the most active form of vitamin
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5 335 D, may increase mycobacterial killing by macrophages but also limits host damage by
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8 336 decreasing the gamma-interferon production [23-28]. In perhaps the strongest evidence to
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10 337 date for a role of vitamin D in tuberculosis, a study by Liu *et al* [29] found that the
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12 338 antimycobacterial response in humans is dependent on adequate availability of vitamin D.

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19 340 A few randomized trials of vitamin D supplementation in TB patients have been
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21 341 conducted in the past few years [4 5 30]. In a randomized trial that was conducted among
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23 342 365 TB patients in Guinea-Bissau starting antituberculosis treatment, overall mortality
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25 343 was 15% (54 of 365) at 1 year of follow-up and similar in both arms [5]. Martineau and
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27 344 colleagues didn't find a difference in median time to sputum culture conversion with
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29 345 vitamin D supplementation of 2.5 mg vitamin D₃ at enrollment, 14, 28, and 42 days after
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31 346 starting TB treatment in 126 adults with sputum smear-positive PTB [4]. A recent report
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33 347 by Coussens *et al* from a subset of the 126 adults included in the trial above stated that
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35 348 median time to sputum smear conversion in the intervention arm was significantly shorter
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37 349 than in the control arm (23 vs. 36 days; p=0.04) [30]. The lack of effect and concordance
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39 350 in most of these trials is probably due to the dose and dosing interval used. It is worth
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41 351 noting however, that large intermittent doses of vitamin D may result in
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43 352 supraphysiological concentrations in some cases, which may be more harmful than
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45 353 helpful in their effects on the immune system [31].
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3 355 Vitamin D insufficiency also was associated with T-cell subset counts only among the
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6 356 HIV-infected patients in this cohort. We can only speculate as to the reasons for the
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8 357 significantly higher increase in CD4 T-cells observed in patients with vitamin D
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10 358 insufficiency at baseline. One potential explanation is that HIV-infected patients with
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12 359 vitamin D insufficiency may experience more uncontrolled immune reconstitution,
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14 360 leading to a greater increase in CD4 T-cell counts, on treatment of TB, compared to
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16 361 patients with adequate vitamin D status. This may also explain why this relationship is
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18 362 observed only in the first eight months of follow-up and not subsequently.
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364 The results for CD8 and CD3 T-cells are consistent with our previous studies among
365 HIV-infected women in Tanzania [13 14]. This could suggest a possible role of vitamin
366 D in inflammation. Although, the conventional role of CD8 cells is as cytotoxic cells,
367 they also are effector cells in inflammation [32]. The involvement of vitamin D in
368 modulating CD8 cells is also indicated by the fact that CD8 cells express the highest
369 concentration of vitamin D receptor of the immune cells [33]. Other studies also have
370 found that vitamin D suppresses antigen-stimulated proinflammatory cytokine responses,
371 which may help speed up resolution of inflammatory responses that can lead to increased
372 risk of mortality among TB patients [30].

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374 TB, once known as 'consumption', is associated with significant wasting and weight loss.
375 The observation that better vitamin D status among HIV-uninfected patients is associated
376 with a greater increase in BMI during follow-up is likely related to decreased risk of

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3 377 relapse among these patients, as well as improvement in quality of life through
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5 378 mechanisms such as better metabolism that were not directly assessed in this study.
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8 379 The major strengths of this study include a large number of participants, more than half
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10 380 of whom were HIV-infected, comprehensive assessment of clinical, immunological,
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12 381 socio-demographic, and nutritional parameters, and a long duration of follow-up. On the
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14 382 other hand, the major limitation is the possibility of reverse causation and residual
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16 383 confounding. We have attempted to minimize this through rigorous analyses and
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18 384 adjusting for several potential confounders, including hemoglobin concentrations, HIV
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20 385 status, viral load, CD4 T-cells, and Karnofsky score, in most analyses. The study results
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22 386 are generalizable to most settings with a high TB burden and widely prevalent vitamin D
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24 387 insufficiency.
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32 389 In summary, our study results indicate that adequate vitamin D status is associated with
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34 390 better clinical and nutritional parameters during follow-up in a cohort of TB patients in
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36 391 Tanzania. While randomized trials of vitamin D supplementation among TB patients are
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38 392 urgently warranted, it is also imperative to conduct dose-response studies to determine
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40 393 ideal dose and duration for the supplement.
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28 407 The authors report NO conflict of interest
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33 409 ***Author Contributions***

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35 410 SM wrote the first draft of the manuscript and analyzed and interpreted the data; FMM,
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37 411 RJB, SA, WU, EV, and WWF were investigators of the parent trial and contributed to
38
39 412 field activities and oversight; RJB also helped with the analysis and interpretation of the
40
41 413 data; all authors participated in study design and contributed to the final manuscript. All
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43 414 authors have also read and approved the final manuscript.
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47 415 ***Data sharing***

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49 416 No additional data available on vitamin D and tuberculosis.
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References

1. WHO. Global Tuberculosis Control. Geneva: World Health Organization, 2011.
2. Chaisson RE, Martinson NA. Tuberculosis in Africa--combating an HIV-driven crisis. *N Engl J Med* 2008;**358**(11):1089-92 doi: 358/11/1089 [pii] 10.1056/NEJMp0800809[published Online First: Epub Date]].
3. Martineau AR, Nhamoyebonde S, Oni T, et al. Reciprocal seasonal variation in vitamin D status and tuberculosis notifications in Cape Town, South Africa. *Proc Natl Acad Sci U S A* 2011;**108**(47):19013-7 doi: 10.1073/pnas.1111825108[published Online First: Epub Date]].
4. Martineau AR, Timms PM, Bothamley GH, et al. High-dose vitamin D(3) during intensive-phase antimicrobial treatment of pulmonary tuberculosis: a double-blind randomised controlled trial. *Lancet* 2011;**377**(9761):242-50 doi: 10.1016/S0140-6736(10)61889-2[published Online First: Epub Date]].
5. Wejse C, Gomes VF, Rabna P, et al. Vitamin D as supplementary treatment for tuberculosis: a double-blind, randomized, placebo-controlled trial. *American Journal of Respiratory and Critical Care Medicine* 2009;**179**(9):843-50 doi: 10.1164/rccm.200804-567OC[published Online First: Epub Date]].
6. Villamor E, Mugusi F, Urassa W, et al. A Trial of the Effect of Micronutrient Supplementation on Treatment Outcome, T Cell Counts, Morbidity, and Mortality in Adults with Pulmonary Tuberculosis. *J Infect Dis* 2008;**197**(11):1499-505 doi: 10.1086/587846 10.1086/587846 [pii][published Online First: Epub Date]].

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7. Karnofsky DA, Abelmann WH, Craver LF, et al. The use of nitrogen mustards in the palliative treatment of cancer. *Cancer* 1948;**1**:634-56
8. Fawzi WW, Msamanga GI, Spiegelman D, et al. A randomized trial of multivitamin supplements and HIV disease progression and mortality. *N Engl J Med* 2004;**351**(1):23-32
9. Baum M, Cassetti L, Bonvehi P, et al. Inadequate dietary intake and altered nutrition status in early HIV-1 infection. *Nutrition* 1994;**10**(1):16-20
10. Lohman TG, Roche AF, Martorell R. *Anthropometric standardization reference manual*. Champaign, IL: Human Kinetics Books, 1988.
11. WHO. Interim proposal for a WHO staging system for HIV infection and disease. *Wkly Epidemiol Rec* 1990;**65**:221-24
12. Friis H, Range N, Pedersen ML, et al. Hypovitaminosis D is common among pulmonary tuberculosis patients in Tanzania but is not explained by the acute phase response. *J Nutr* 2008;**138**(12):2474-80 doi: 10.1093/ajph/98.12.2474 [pii] 10.3945/jn.108.094979[published Online First: Epub Date].
13. Mehta S, Giovannucci E, Mugusi FM, et al. Vitamin D status of HIV-infected women and its association with HIV disease progression, anemia, and mortality. *PLoS ONE* 2010;**5**(1):e8770 doi: 10.1371/journal.pone.0008770[published Online First: Epub Date].
14. Mehta S, Spiegelman D, Aboud S, et al. Lipid-soluble vitamins A, D, and E in HIV-infected pregnant women in Tanzania. *European journal of clinical nutrition* 2010;**64**(8):808-17 doi: 10.1038/ejcn.2010.76[published Online First: Epub Date].
15. Davies PD, Church HA, Bovornkitti S, et al. Altered vitamin D homeostasis in tuberculosis. *Intern Med (Thailand)* 1988;**4**:45-47

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16. Norman AW. Nutritional Aspects of Vitamin D. <http://vitamind.ucr.edu/nutri.html>. Accessed Oct 9, 2006. 1999. <http://vitamind.ucr.edu/nutri.html> (accessed Oct 9, 2006).
17. Utiger RD. The need for more vitamin D. *N Engl J Med* 1998;**338**(12):828-9
18. Holick MF. High prevalence of vitamin D inadequacy and implications for health. *Mayo Clin Proc* 2006;**81**(3):353-73
19. Greenland S. Modeling and variable selection in epidemiologic analysis. *Am J Public Health* 1989;**79**(3):340-9
20. Mehta S, Mugusi FM, Spiegelman D, et al. Vitamin D status and its association with morbidity including wasting and opportunistic illnesses in HIV-infected women in Tanzania. *AIDS Patient Care STDS* 2011;**25**(10):579-85 doi: 10.1089/apc.2011.0182[published Online First: Epub Date].
21. Sudfeld CR, Giovannucci EL, Isanaka S, et al. Vitamin D Status and Incidence of Pulmonary Tuberculosis, Opportunistic Infections, and Wasting Among HIV-Infected Tanzanian Adults Initiating Antiretroviral Therapy. *The Journal of infectious diseases* 2013;**207**(3):378-85 doi: 10.1093/infdis/jis693[published Online First: Epub Date].
22. Sudfeld CR, Wang M, Aboud S, et al. Vitamin D and HIV progression among Tanzanian adults initiating antiretroviral therapy. *PloS one* 2012;**7**(6):e40036 doi: 10.1371/journal.pone.0040036[published Online First: Epub Date].
23. Thoma-Uszynski S, Stenger S, Takeuchi O, et al. Induction of direct antimicrobial activity through mammalian toll-like receptors. *Science* 2001;**291**(5508):1544-7
24. Waters WR, Palmer MV, Nonnecke BJ, et al. Mycobacterium bovis infection of vitamin D-deficient NOS2^{-/-} mice. *Microb Pathog* 2004;**36**(1):11-7

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25. Waters WR, Nonnecke BJ, Foote MR, et al. Mycobacterium bovis bacille Calmette-Guerin vaccination of cattle: activation of bovine CD4+ and gamma delta TCR+ cells and modulation by 1,25-dihydroxyvitamin D3. *Tuberculosis (Edinb)* 2003;**83**(5):287-97
26. Waters WR, Nonnecke BJ, Rahner TE, et al. Modulation of Mycobacterium bovis-specific responses of bovine peripheral blood mononuclear cells by 1,25-dihydroxyvitamin D(3). *Clin Diagn Lab Immunol* 2001;**8**(6):1204-12
27. Kawakami K, Teruya K, Tohyama M, et al. [A therapeutic trial of experimental tuberculosis with gamma-interferon in an immunocompromised mouse model]. *Kekkaku* 1994;**69**(10):607-13
28. McMurray DN, Bartow RA, Mintzer CL, et al. Micronutrient status and immune function in tuberculosis. *Ann N Y Acad Sci* 1990;**587**:59-69
29. Liu PT, Stenger S, Li H, et al. Toll-Like Receptor Triggering of a Vitamin D-Mediated Human Antimicrobial Response. *Science* 2006:1123933 doi: 10.1126/science.1123933[published Online First: Epub Date].
30. Coussens AK, Wilkinson RJ, Hanifa Y, et al. Vitamin D accelerates resolution of inflammatory responses during tuberculosis treatment. *Proc Natl Acad Sci* 2012:Epub
31. Martineau AR. Bolus-dose vitamin D and prevention of childhood pneumonia. *Lancet* 2012;**379**(9824):1373-5 doi: 10.1016/S0140-6736(12)60405-X[published Online First: Epub Date].
32. Babbe H, Roers A, Waisman A, et al. Clonal expansions of CD8(+) T cells dominate the T cell infiltrate in active multiple sclerosis lesions as shown by micromanipulation and single cell polymerase chain reaction. *J Exp Med* 2000;**192**(3):393-404

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2
3 33. Veldman CM, Cantorna MT, DeLuca HF. Expression of 1,25-dihydroxyvitamin D(3)
4 receptor in the immune system. Arch Biochem Biophys 2000;374(2):334-8 doi:
5
6 10.1006/abbi.1999.1605
7
8 S0003-9861(99)91605-3 [pii][published Online First: Epub Date].
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Figure Legends

1. Distribution of 25-hydroxyvitamin D concentrations at baseline (nmol/L)
2. Distribution of 25-hydroxyvitamin D concentrations by season of blood draw;
Season 1: Dry (January-February); Season 2: Long Rains (March-June); Season 3:
Dry (July-October); Season 4: Short Rains (November-December)

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Tables

Table 1	Baseline Characteristics of the Study Population (n=677)		
		HIV-infected (n=344)	HIV-uninfected (n=333)
Variable		Mean (Standard Deviation)	Mean (Standard Deviation)
Age, years		34.4 ± 8.6	30.2 ± 9.2
Money spent on food per person per day, Tanzanian Shillings*		587.3 ± 445.9	580.1 ± 684.2
Hemoglobin, g/dL		9.9 ± 1.8	11.1 ± 1.7
Albumin, g/dL		2.8 ± 1.0	3.2 ± 1.1
CD3 T-cell count, cells/μL		1228.0 ± 608.5	1195.9 ± 404.8
CD4 T-cell counts, cells/μL		327.2 ± 246.2	709.2 ± 250.8
CD8 T-cell counts, cells/μL		826.9 ± 447.5	427.5 ± 188.2
Log(10) Viral Load, copies/mL		4.6 ± 1.0	N/A
Body Mass Index, kg/m ²		19.4 ± 2.8	18.8 ± 2.5
Mid-Upper Arm Circumference, cm		23.4 ± 2.7	23.1 ± 2.7
Follow-up time, days		916.8 ± 507.4	1532.9 ± 331.4
		n (%)	n (%)
Vitamin D insufficiency (serum 25- hydroxyvitamin D <75 nmol/L)		218 (63.4%)	200 (60.1%)

	Vitamin D deficiency (serum 25-hydroxyvitamin D <50 nmol/L)	55 (16.0%)	51 (15.3%)
	Sex		
	Male	203 (59.0%)	257 (77.2%)
	Female	141 (41.0%)	76 (22.8%)
	Center		
	Mwananyamala	79 (23.0%)	88 (26.4%)
	Temeke	102 (29.7%)	83 (24.9%)
	Tandale	83 (24.1%)	91 (27.3%)
	Mbgala	31 (9.0%)	70 (21.0%)
	Amana	49 (14.2%)	1 (.3%)
	Karnofsky Score <70%	45 (13.1%)	29 (8.7%)
	Education Group		
	None	29 (8.4%)	36 (10.8%)
	Low <5years	35 (10.2%)	31 (9.3%)
	Primary 5-8 years	238 (69.2%)	233 (70.0%)
	Secondary/University	42 (12.2%)	33 (9.9%)

	Cohabits with a Partner	200 (58.1%)	168 (50.5%)
	Assets at home		
	None	92 (26.9%)	108 (32.4%)
	One	89 (26.0%)	85 (25.5%)
	2-3	122 (35.7%)	114 (34.2%)
	4-5	39 (11.4%)	26 (7.8%)
	WHO HIV Disease Stage		
	3	240 (90.9%)	N/A
	4	24 (9.1%)	
	CD4 T-cell categories, cells/ μ L		
	0-199	97 (35.9%)	0 (.0%)
	200-499	116 (43.0%)	69 (22.9%)
	500+	57 (21.1%)	232 (77.1%)
	WHO BMI Group, kg/m ²		
	<16	26 (7.7%)	33 (9.9%)
	16-16.99	37 (10.9%)	45 (13.6%)
	17-18.49	73 (21.5%)	88 (26.5%)
	18.5-19.99	79 (23.3%)	70 (21.1%)
	20-21.99	77 (22.7%)	69 (20.8%)

	22+	47 (13.9%)	27 (8.1%)
* 1 US Dollar \cong 1000 Tanzanian Shillings at the time of the study			

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Table 2		Vitamin D Status and Mortality and HIV Disease Progression in HIV-infected TB Patients			
		Univariate		Multivariate	
Outcome	n/N (%)	RR (95% CI)	p-value	RR (95% CI)	p-value
Mortality					
Vitamin D insufficiency (<75 nmol/L)	61/218 (28.0%)	0.73 (0.50, 1.08)	0.12	0.70 (0.47, 1.04)	0.08
Adequate Vitamin D	43/126 (34.1%)				
Vitamin D deficient (<50 nmol/L)	20/55 (36.4%)	1.34 (0.82, 2.18)	0.25	0.91 (0.55 1.50)	0.71
Not deficient	84/289 (29.1%)				
Continuous Vitamin D (nmol/L)		1.00 (0.99, 1.01)	0.49	1.01 (1.00, 1.02)	0.15
HIV Disease Progression					

Vitamin D insufficiency (<75 nmol/L)	46/150 (30.7%)	1.10 (0.67, 1.82)	0.71	1.08 (0.64, 1.82)	0.78
Adequate Vitamin D	23/90 (25.6%)	Reference		Reference	
Vitamin D deficient (<50 nmol/L)	14/34 (41.2%)	1.91 (1.05, 3.44)	0.03	1.48 (0.78, 2.82)	0.23
Not deficient	55/206 (26.7%)	Reference		Reference	
Continuous Vitamin D (nmol/L)		0.99 (0.98, 1.01)	0.30	1.00 (0.99, 1.01)	0.57
p-values obtained using Cox Proportional Hazards Regression; RR: Risk Ratio; 95% CI: 95% Confidence Interval					
Multivariate analyses adjusted for Age, Karnofsky Score, Baseline Hemoglobin, Viral Load, HIV Status, CD4 Counts, and Micronutrient Supplementation					

Table 3		Vitamin D Status and Treatment Outcomes in TB Patients				
		Univariate			Multivariate	
Outcome	n/N (%)	RR (95% CI)	p-value	RR (95% CI)	p-value	
Treatment Failure by 1 month post-treatment initiation						
Vitamin D insufficiency (<75 nmol/L)	58/298 (19.5%)	1.06 (0.72, 1.55)	0.77	1.02 (0.70, 1.49)	0.93	
Adequate Vitamin D	34/185 (18.4%)					
Vitamin D deficient (<50 nmol/L)	15/75 (20.0%)	1.06 (0.65, 1.74)	0.82	1.13 (0.69, 1.86)	0.63	
Not deficient	77/408 (18.9%)					
Continuous Vitamin D (nmol/L)		1.00 (0.99, 1.01)	0.49	1.00 (0.99, 1.01)	0.50	
Any Relapse (relapse after 1 month post-treatment initiation if culture negative at 1 month)						
Vitamin D insufficiency	51/227 (22.5%)	1.56 (0.98, 2.48)	0.06	1.66 (1.04, 2.64)	0.03	

	(<75 nmol/L)					
	Adequate Vitamin D	21/146 (14.4%)				
	Vitamin D deficient (<50 nmol/L)	13/56 (23.2%)	1.25 (0.73, 2.12)	0.41	1.40 (0.82, 2.39)	0.21
	Not deficient	59/317 (18.6%)				
	Continuous Vitamin D (nmol/L)		0.99 (0.98, 1.00)	0.06	0.99 (0.98, 1.00)	0.04
p-values obtained using Binomial Regression; RR: Risk Ratio; 95% CI: 95% Confidence Interval						
Multivariate analyses adjusted for Age, Karnofsky Score, Baseline Hemoglobin, Viral Load, HIV Status, CD4 Counts, and Micronutrient Supplementation						

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Table 4	Vitamin D Status and T-cell Counts (cells/ μ L) in TB Patients											
	CD4 T-cells					CD8 T-cells					CD3 T-cells	
Outcome	Adequate vitamin D, mean difference (SD) ^a	Vitamin D insufficiency, mean difference (95% CI) ^b	Vitamin D insufficiency, adjusted mean difference (95% CI) ^c	p-value		Adequate vitamin D, mean difference (SD) ^a	Vitamin D insufficiency, adjusted mean difference (95% CI) ^b	Vitamin D insufficiency, adjusted mean difference (95% CI) ^c	p-value		Adequate vitamin D, mean difference (SD) ^a	Vitamin D insufficiency, adjusted mean difference (95% CI) ^c

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Entire follow-up: HIV-infected patients													
Vitamin D insufficiency (<75 nmol/L)	300 (234)	17 (-23, 56)	21 (-18, 59)	0.2 9	902 (457)	88 (7, 169)	85 (4, 165)	0.0 4	1298 (635)	101 (-4, 206)	103 (-5, 212)	0.0 6	
Vitamin D deficient (<50 nmol/L)	333 (225)	21 (-34, 76)	30 (-26, 86)	0.2 9	957 (424)	105 (-9, 219)	114 (-6, 234)	0.0 6	1392 (595)	104 (-47, 255)	125 (-28, 279)	0.1 1	
Continuous Vitamin D (per nmol/L)		0 (-1, 1)	-1 (-1, 0)	0.2 6		-3 (-5, -1)	-3 (-5, -1)	0.0 04		-3 (-5, -1)	-3 (-6, -1)	0.0 1	
Entire follow-up: HIV-uninfected patients													
Vitamin D	771	-2 (-)	3 (-45, 3)	0.9	508	-25 (-)	-22 (-)	0.2	1351	-37 (-)	-28 (-)	0.4	

	insufficiency (<75 nmol/L)	(235)	49, 45)	51)	1	(209)	63, 14)	60, 17)	7	(400)	109, 35)	99, 44)	5
	Vitamin D deficient (<50 nmol/L)	781 (241)	-34 (-99, 30)	-34 (-101, 32)	0.3 1	500 (195)	-1 (-64, 62)	3 (-61, 67)	0.9 3	1354 (397)	-33 (-136, 71)	-28 (-134, 79)	0.6 1
	Continuous Vitamin D (per nmol/L)		0 (-1, 1)	0 (-1, 1)	0.9 7		0 (-1, 1)	0 (-1, 1)	0.8 3		0 (-1, 2)	0 (-2, 2)	0.9 0
First 8 months of follow-up: HIV-infected patients													
	Vitamin D insufficiency (<75 nmol/L)	316 (237)	54 (8, 100)	58 (13, 104)	0.0 1	868 (470)	132 (29, 235)	119 (15, 223)	0.0 2	1279 (670)	190 (42, 337)	179 (28, 331)	0.0 2

	Vitamin D deficient (<50 nmol/L)	372 (264)	36 (-25, 97)	41 (-20, 101)	0.1 9	963 (471)	63 (-77, 203)	75 (-72, 221)	0.3 2	1443 (689)	67 (-125, 259)	101 (-93, 295)	0.3 1
	Continuous Vitamin D (per nmol/L)		-1 (-2, 0)	-1 (-2, 0)	0.0 1		-4 (-6, -1)	-4 (-6, -1)	0.0 02		-4 (-7, -1)	-5 (-8, -2)	0.0 03
First 8 months of follow-up: HIV-uninfected patients													
	Vitamin D insufficiency (<75 nmol/L)	724 (243)	1 (-52, 53)	6 (-47, 59)	0.8 2	461 (232)	-22 (-63, 20)	-17 (-57, 22)	0.3 9	1248 (446)	-38 (-121, 46)	-27 (-106, 52)	0.5 0
	Vitamin D deficient	731	-7 (-	-7 (-	0.8	454	4 (-73,	5 (-71,	0.9	1247	14 (-	17 (-	0.8

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	(<50 nmol/L)	(237)	95, 80)	96, 81)	7	(209)	81)	81)	0	(410)	124, 153)	123, 156)	2
	Continuous Vitamin D (per nmol/L)		0 (-2,1)	0 (-2,1)	0.5 4		0 (-1, 1)	0 (-1, 1)	0.7 3		0 (-2, 1)	0 (-2, 1)	0.5 7

^a Data are the means (SD) of the average measurement during follow-up for each participant

^b Data are the mean difference between the low and the adequate vitamin D group, as defined in Column B. The mean differences, 95% confidence intervals (CIs), and corresponding p-values were estimated from generalized estimating equations, after adjustment for baseline measurements, follow-up time, and treatment (micronutrients vs. placebo) group.

^c Multivariate analyses additionally adjusted for Age, Karnofsky Score, and Baseline Hemoglobin

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Table 5 Vitamin D Status and Nutritional Parameters in TB Patients												
	Body Mass Index (kg/m ²)				Albumin concentration (g/dL)				Hemoglobin concentration (g/dL)			
Outcome	Adequate vitamin D, mean (SD) ^a	Vitamin D insufficiency, mean difference (95% CI) ^b	Vitamin D insufficiency, adjusted mean difference (95% CI) ^c	p-value	Adequate vitamin D, mean (SD) ^a	Vitamin D insufficiency, mean difference (95% CI) ^b	Vitamin D insufficiency, adjusted mean difference (95% CI) ^c	p-value	Adequate vitamin D, mean (SD) ^a	Vitamin D insufficiency, mean difference (95% CI) ^b	Vitamin D insufficiency, adjusted mean difference (95% CI) ^c	p-value
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follow-up:													
All patients													
Vitamin D insufficiency (<75 nmol/L)	21.20 (2.80)	-0.06 (-0.30, 0.17)	-0.08 (-0.30, 0.14)	0.46	3.42 (0.74)	-0.05 (-0.14, 0.04)	0.00 (-0.08, 0.08)	0.9 7	12.65 (1.80)	-0.16 (-0.40, 0.08)	-0.18 (-0.41, 0.05)	0 12	
Vitamin D deficient (<50 nmol/L)	21.23 (3.00)	-0.16 (-0.46, 0.14)	-0.14 (-0.44, 0.15)	0.34	3.42 (0.72)	-0.05 (-0.17, 0.07)	0.02 (-0.08, 0.13)	0.65	12.42 (1.87)	0.15 (-0.16, 0.45)	0.17 (-0.11, 0.45)	0.2 4	

	Continuous Vitamin D (per nmol/L)	0.00 (0.00, 0.01)	0.00 (0.00, 0.01)	0.30			0.002 (0.00, 0.004)	0.000 (-0.002, 0.002)	0.90		0.00 (-0.01, 0.01)	0.00 (0.00, 0.01)	0.8 5	
For peer review only														
	First 8 months of follow-up: All patients													
	Vitamin D insufficiency (<75 nmol/L)	20.96 (2.73)	-0.20 (-0.40, -0.01)	-0.21 (-0.39, -0.02)	0.03		3.42 (1.09)	-0.01 (-0.18, 0.16)	0.04 (-0.13, 0.21)	0.65	12.12 (1.85)	-0.01 (-0.28, 0.26)	-0.04 (-0.31, 0.23)	0.7 8

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Vitamin D deficient (<50 nmol/L)	20.85 (2.84)	0.00 (-0.25, 0.25)	0.04 (-0.21, 0.29)	0.78	3.41 (1.08)	-0.11 (-0.32, 0.10)	-0.05 (-0.27, 0.17)	0.64		11.92 (1.99)	0.16 (-0.18, 0.50)	0.21 (-0.10, 0.53)	0.1 9	
Continuous Vitamin D (per nmol/L)		0.00 (0.00, 0.01)	0.00 (0.00, 0.01)	0.38		0.000 (-0.003, 0.005)	0.000 (-0.004, 0.004)	0.87			0.00 (-0.01, 0.00)	0.00 (-0.01, 0.00)	0.5 7	

^aData are the means (SD) of the average measurement during follow-up for each participant

^bData are the mean difference between the low and the adequate vitamin D group, as defined in Column B. The mean differences, 95% confidence intervals (CIs), and corresponding p-values were estimated from generalized estimating equations, after adjustment for baseline

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5 measurements, follow-up time, and treatment (micronutrients vs. placebo) group.
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7 ^c Multivariate analyses additionally adjusted for Age, Karnofsky Score, Baseline Hemoglobin, Viral Load, CD4 Count, and HIV Status;
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9 HIV status removed from the model where the results are stratified by HIV status. Viral Load also removed from the model in HIV-
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11 uninfected individuals.
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For peer review only

Vitamin D Status and TB Treatment Outcomes in Adult Tanzanian Patients

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2
3 1 *Abstract*

4 2 **Objectives:** Vitamin D is an immunomodulator and can alter response to tuberculosis
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6 treatment, though randomized trials have been inconclusive to date. We present the first
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8 comprehensive analysis of the associations between vitamin D status and TB treatment,
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10 T-cell counts, and nutritional outcomes by HIV status.
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17 7 **Design:** Cohort study
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22 9 **Setting:** Outpatient clinics in Tanzania
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27 11 **Participants:** 25-hydroxyvitamin D levels were assessed in a cohort of 677 patients with
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29 TB (344 HIV-infected) initiating anti-TB treatment at enrollment in a multivitamin
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31 supplementation (excluding vitamin D) trial (Clinicaltrials.gov identifier:
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33 [NCT00197704](#)).
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39 16 **Primary and secondary outcome measures:** Information on treatment outcomes such as
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41 failure and relapse, HIV disease progression, T-cell counts, and anthropometry was
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43 collected routinely, with a median follow-up of 52 and 30 months for HIV-uninfected and
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45 HIV-infected patients, respectively. Cox and binomial regression, and generalized
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47 estimating equations were used to assess the association of vitamin D status with these
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49 outcomes.
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4 23 **Results:** Mean 25-hydroxyvitamin D concentrations at enrollment were 69.8 (\pm 21.5)
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6 24 nmol/L [27.9 (\pm 8.6) ng/mL]. Vitamin D insufficiency (<75 nmol/L) was associated with
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8 25 a 66% higher risk of relapse (95% CI: 4%, 164%; 133% higher risk in HIV-uninfected
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10 26 patients). Each unit higher vitamin D levels at baseline were associated with a decrease of
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12 27 3 ($p=0.004$) CD8 and 3 ($p=0.01$) CD3 T-cells/ μ L during follow-up in HIV-infected
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14 28 patients. Vitamin D insufficiency was also associated with a greater decrease of BMI (-
15
16 29 0.21 kg/m²; 95% CI:-0.39, -0.02), during the first eight months of follow-up. No
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18 30 association was observed for vitamin D status with mortality or HIV disease progression.
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22 32 **Conclusions:** Adequate vitamin D status is associated with a lower risk of relapse and
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24 33 with improved nutritional indicators such as BMI in TB patients, with or without HIV
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26 34 infection. Further research is needed to determine the optimal dose of vitamin D and
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28 35 effectiveness of daily vitamin D supplementation among patients with TB.
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3 38 **Article Focus:** Recent laboratory data has suggested that optimal vitamin D status may
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5 39 be associated with a more effective immune response to TB infection, a faster rate of
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8 40 bacteriologic cure, and better long-term outcomes. However, clinical and epidemiological
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11 41 studies have found inconsistent results. In this paper, we present the first comprehensive
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13 42 analysis of the associations between vitamin D status and TB treatment, T-cell counts,
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15 43 and nutritional outcomes by HIV status.
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20 45 **Key Messages:** We found that patients with adequate vitamin D status were less likely to
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22 46 experience a relapse during follow-up after completing TB treatment. They were also
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24 47 more likely to have a better nutritional status, as assessed by their body mass index,
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27 48 during follow-up, compared to patients with vitamin D insufficiency. The results provide
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29 49 justification for conducting both a dose response study to determine optimal dose of
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31 50 vitamin D and a randomized controlled trial of daily vitamin D supplementation among
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34 51 patients with TB.
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39 53 **Strengths and Limitations of this study:** The major strengths of this study include a
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41 54 large number of participants, more than half of whom were HIV-infected, comprehensive
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43 55 assessment of clinical, immunological, socio-demographic, and nutritional parameters,
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46 56 and a long duration of follow-up. On the other hand, the major limitation is the possibility
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48 57 of reverse causation and residual confounding. We have attempted to minimize this
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51 58 through rigorous analyses and adjusting for several potential confounders, including
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53 59 hemoglobin concentrations, HIV status, viral load, CD4 T-cells, and Karnofsky score, in
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55 60 most analyses.
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3 61 **Introduction**
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5 62 *Mycobacterium tuberculosis* is one of the most pernicious infectious diseases and
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8 63 successful pathogens known to man. More than 95% of the estimated cases and deaths
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10 64 due to tuberculosis (TB) occur in low-income countries. The United Republic of
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12 65 Tanzania is one of the 22 high-burden countries that account for 80% of global TB cases.
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14 66 Tanzania has an incidence of 177 cases per 100,000 population per year and a prevalence
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16 67 of 183 cases per 100,000 population per year [1].
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22 69 The spread of Human Immunodeficiency Virus (HIV) has fuelled the resurgence of the
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24 70 TB epidemic in Tanzania, as in other parts of sub-Saharan Africa [2]. HIV is the
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26 71 strongest factor in the development of active TB; it is estimated that only one out of ten
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28 72 immunocompetent persons infected with TB develops active TB in his/her lifetime;
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30 73 whereas, one out of ten HIV-infected persons infected with TB will develop active TB
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32 74 every year. An estimated 38% of TB patients in Tanzania are also infected with HIV [1].
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38 76 Current treatment regimens, given under appropriate management conditions, are nearly
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40 77 100% curative for patients with drug-susceptible organisms. However, in Tanzania,
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42 78 treatment fails in 12-17% of the cases. Additionally, TB patients in settings such as
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44 79 Tanzania grapple with multiple health-related and quality of life issues, which are not
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46 80 addressed adequately with treatment alone.
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52 82 Recent data has suggested that optimal vitamin D status may be associated with a more
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54 83 effective immune response to TB infection, a faster rate of bacteriologic cure, and better
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3 84 long-term outcomes. For example, a recent cross-sectional study found that vitamin D
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5 85 deficiency is highly prevalent in South Africa and is associated with susceptibility to
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8 86 active TB both in the presence and absence of HIV infection [3]. A few randomized trials
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10 87 have also been conducted; two of the recent ones failed to find an effect of vitamin D
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12 88 supplementation on treatment success [4 5]. However, the dose used and duration of
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14 89 supplementation may have precluded finding an effect. Further, most studies had small
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16 90 sample sizes and assessed only a limited number of covariates.
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22 92 In this manuscript, we comprehensively examined the hypotheses that vitamin D status
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24 93 may be associated with response to treatment, risk of treatment failure, laboratory
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26 94 parameters such as T-cell counts, and anthropometric measurements in the context of a
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28 95 randomized trial of micronutrient supplementation (supplement did not contain vitamin
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30 96 D) in Tanzania to better inform future studies or trials.
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34 97 35 36 98 ***Materials and Methods***

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38 99 Study Population: The study population and recruitment methods have been described in
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40 100 detail earlier [6]. Briefly, 887 adults with pulmonary tuberculosis (PTB) were enrolled in
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42 101 a randomized trial (Clinicaltrials.gov identifier: NCT00197704) to examine the effects of
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44 102 micronutrient supplementation on TB treatment failure, relapse, and mortality. The trial
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46 103 started in April 2000 in Dar es Salaam, Tanzania and continued until April 2005. The
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48 104 eligibility criteria for the study included positive sputum smears for acid-fast bacilli
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50 105 (AFB), age between 18 and 65 years, Karnofsky performance score of $\geq 40\%$ [7], plan to
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52 106 stay in Dar es Salaam for 2 years, not being pregnant, and not having received anti-TB
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3 107 treatment during the previous one year. Consenting subjects were randomly assigned in
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5 108 computer-generated permuted blocks of 20, stratified by HIV status, to receive a daily
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8 109 oral dose of 1 of 2 regimens: micronutrients (5000 IU of retinol, 20 mg of vitamin B1,
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10 110 20mg of vitamin B2, 25mg of vitamin B₆, 100 mg of niacin, 50 µg of vitamin B₁₂, 500
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12 111 mg of vitamin C, 200 mg of vitamin E, 0.8 mg of folic acid, and 100 µg of selenium) or
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14 112 placebo. These doses represent between 6 and 10 times the recommended dietary
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16 113 allowance (RDA) and were being tested at the time among HIV-infected adults from this
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18 114 setting [8]. We chose multiples of the RDA because previous observational studies
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20 115 suggested that HIV-infected individuals need higher dietary intakes of micronutrients to
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22 116 achieve normal serum concentrations [9]. All patients received a daily combination of
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24 117 rifampicin, isoniazid, pyrazinamide, and ethambutol under direct observation of a health
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26 118 worker during the first 2 months (intensive phase) followed by 6 months of self-
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28 119 administered daily isoniazid and ethambutol, as per the Tanzania National TB and
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30 120 Leprosy Programme guidelines. None of the HIV-infected patients received antiretroviral
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32 121 therapy, as these medications were not routinely available in Tanzania at the time this
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34 122 trial was conducted.
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43 124 At the time of randomization, research nurses collected information on various socio-
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45 125 demographic characteristics including age, education levels, marital status, and
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47 126 socioeconomic status. Anthropometric measurements were also obtained using
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49 127 standardized procedures [10] at the randomization visit as well as during each monthly
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51 128 follow-up visit. Height was measured to the nearest 0.1 cm using SECA Bodymeter 206
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53 129 stadiometers, weight to the nearest 100 g with SECA 700 balance beam scales, and left
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3 130 mid-upper arm circumference (MUAC) at the midpoint between the acromion and
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5 131 olecranon to the nearest 0.1 cm using non-stretchable tailor's tapes.
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10 133 Physician visits were scheduled every 3 months. During these visits, study physicians
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12 134 inquired about the health of the subject during the preceding period and performed a
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14 135 complete physical examination. The stage of HIV disease was assessed according to the
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16 136 World Health Organization system [11].
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22 138 Ethics Approval: A written informed consent was obtained from all the study
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24 139 participants. The institutional review boards of the Muhimbili University of Health and
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26 140 Allied Sciences, the Tanzanian National AIDS Control Program, and the Harvard School
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28 141 of Public Health approved the study protocol.
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34 143 Laboratory Methods: At the time of initiation of anti-TB treatment, HIV status was
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36 144 assessed among consenting patients using 2 sequential ELISAs (Wellcozyme, Murex
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38 145 Biotech; Enzygnost anti-HIV1/2 Plus, Dade Behring); discrepant results were resolved by
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40 146 Western Blot test (Bio-rad, Genetic Systems). Both pre-test and post-test counseling was
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42 147 provided. A blood sample also was obtained for measurement of hemoglobin and
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44 148 albumin concentrations using AcT Diff II hematology analyzer (Beckman Coulter,
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46 149 Miami) and Hitachi 911 analyzer (Roche Diagnostics), respectively. CD4, CD3, and CD8
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48 150 T-cell counts were determined using FACScout or FACSCan systems (Becton
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50 151 Dickinson, CA, USA). Viral load was also determined using the Amplicor HIV-1
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52 152 monitor v1.5 assay (Roche Molecular Systems, Branchburg, NJ, USA).
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6 154 Assessment of Vitamin D Status: Serum 25-hydroxyvitamin D concentrations were
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8 155 measured using liquid chromatography-mass spectrometry at the Children's Hospital in
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10 156 Boston only at enrollment **before the initiation of micronutrient supplementation**. We
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12 157 defined vitamin D insufficiency status as serum 25(OH)D levels of less than 75 nmol/L
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14 158 and adequate otherwise. Vitamin D deficiency was defined as serum 25(OH)D levels of
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16 159 less than 50 nmol/L.
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22 161 Statistical Analysis: We examined the association of vitamin D status with TB treatment
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24 162 outcomes as well as nutritional, immunological, and clinical end points in the entire
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26 163 cohort and separately by HIV status at baseline. TB-related end points included treatment
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28 164 failure, early relapse, and late relapse. Treatment failure by 1 month was defined as
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30 165 positive AFB cultures at 1 month from the initiation of treatment. Relapses were deemed
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32 166 to have occurred in patients with positive cultures, among those who had become culture
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34 167 negative after treatment initiation. Relapses/recurrences included both endogenous
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36 168 reactivation and exogenous reinfection, which could not be distinguished in this study.
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38 169 We calculated the relative risks (RRs) and 95% confidence intervals (CIs) for each of
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40 170 these outcomes by vitamin D status using binomial regression. We used Cox proportional
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42 171 hazards models to assess the association of vitamin D status with mortality in all patients
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44 172 and HIV disease progression from stage 3 to 4 in HIV-infected participants. We defined
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46 173 the end of follow-up as the date when HIV stage was last assessed.
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3 175 We examined the association of vitamin D status with CD4, CD8, and CD3 T-cell counts,
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5 176 viral load (in HIV-infected participants), indicators of nutritional status (body mass index
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7 [BMI] and albumin concentrations), and hemoglobin concentrations using generalized
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9 177
10 178 estimating equations (GEEs). These models do not require that all patients have the same
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12 179 number of follow-up assessments or that the follow-up measurements be obtained at
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14 180 exactly the same time points. We assumed a standard normal distribution for repeated
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16 181 continuous end points (T cell subsets, \log_{10} viral load, anthropometry, and albumin and
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18 182 hemoglobin concentrations) and estimated average differences during follow-up by
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20 183 vitamin D status. We used an exchangeable correlation structure to account for within-
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22 184 subject correlations and adjusted the models for the follow-up time when the
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24 185 measurements had been obtained and for the baseline values.
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32 187 We analyzed the data for the entire period and for the first 8 months, coinciding with the
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34 188 expected end of TB treatment. Multivariate analyses adjusted for age, Karnofsky score,
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36 189 baseline hemoglobin concentrations, viral load, HIV status, CD4 T-cell counts, and
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38 190 micronutrient supplementation, unless otherwise specified in the results section or the
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40 191 tables. All analyses were performed using SAS software version 9.3 (SAS Institute Inc.,
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42 192 Cary NC).
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48 194 **Results**

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50 195 Baseline 25-hydroxyvitamin D concentrations were available for 677 patients out of the
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52 196 original cohort of 887. Mean 25-hydroxyvitamin D concentration was 69.8 (± 21.5)
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54 197 nmol/L [27.9 (± 8.6) ng/mL] and its distribution is shown in Figure 1. The baseline
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3 198 characteristics of these 677 patients by HIV status are presented in Table 1. 36% of the
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5 199 HIV-infected patients had CD4 T-cell counts below 200 cells/ μ L. The mean body mass
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7 200 index (BMI) was 19.1 ± 2.7 kg/m². The median follow-up time for HIV-uninfected
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9 201 patients was 52 months (inter-quartile range [IQR]: 47-57 months) and for HIV-infected
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11 202 patients was 30 months (IQR: 15-41 months).
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18 204 The mean 25-hydroxyvitamin D concentrations were significantly different across season
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20 205 of blood draw in this cohort (p=0.004). Tanzania has four seasons: dry (January-
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22 206 February); long rains (March-June); dry (July-October); short rains (November-
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24 207 December). The boxplot of vitamin D's association with season of blood draw is
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26 208 presented in Figure 2. In subgroup analyses, this association was only observed among
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28 209 the HIV-uninfected patients and not the HIV-infected patients.
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34 211 We examined the correlates of vitamin D insufficiency, defined as serum 25(OH)D
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36 212 concentrations below 75 nmol/L (75 nmol/L) in Supplemental Tables 1 (HIV-uninfected)
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38 213 and 2 (HIV-infected). All factors that had univariate associations with p<0.20 were
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40 214 included in a multivariate model; only the factors that had p<0.05 were retained in the
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42 215 final model. Among the HIV-uninfected subset, patients enrolled in the dry winter season
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44 216 between July and October were 50% more likely to have vitamin D insufficiency,
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46 217 compared to patients enrolled in the dry summer season between January and February (p
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48 218 for season=0.002). Similarly, the participants with the lowest height were more likely to
49
50 219 have vitamin D insufficiency (p=0.01). Finally, greater expenditure on food per person
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52 220 per day was associated with a lower risk of having inadequate vitamin D status (Risk
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3 221 Ratio [RR] per 1000 Tanzanian Shillings [approximately 1 US Dollar at the time of the
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5 222 study]: 0.76; 95% Confidence Interval [CI]: 0.59, 0.98). In the HIV-infected subset,
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7 223 patients with higher hemoglobin concentrations at baseline were less likely to have
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9 224 vitamin D insufficiency, with a 7% lower risk per 1 g/dL higher hemoglobin level
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11 225 ($p=0.007$). On the other hand, higher number of CD4 T-cells was associated with a higher
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13 226 risk of having inadequate vitamin D status (4% higher risk per 100 CD4 T-cells/ μL ;
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15 227 $p=0.02$).

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21 229 There was no significant association of vitamin D status at TB treatment initiation with
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23 230 mortality or HIV disease progression in this cohort (Table 2 includes only HIV-infected
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25 231 participants as there were only 13 deaths in the HIV-uninfected subset). There was no
26
27 232 association observed between vitamin D status and treatment failure one month after
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29 233 initiation of TB treatment (Table 3). However, patients with vitamin D insufficiency (<75
30
31 234 nmol/L) had a 66% higher risk of relapse after becoming culture-negative at one month
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33 235 after initiation of TB treatment (95% CI: 4%, 164%). This association was more
34
35 236 pronounced in those who were not HIV-infected at enrollment in the study (RR: 2.33;
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37 237 95% CI: 1.26, 4.29). In analysis with continuous vitamin D levels, each nmol/L increase
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39 238 was associated with a 1% lower risk of relapse during follow-up ($p=0.04$).

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43 240 Vitamin D insufficiency was observed to have no association with CD4 T-cell counts
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45 241 during the entire follow-up in either the HIV-infected or the HIV-uninfected subsets
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47 242 (Table 4). However, vitamin D insufficiency was associated with greater CD4 T-cell
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3 243 counts during the first eight months of follow-up in the HIV-infected patients (mean
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5 244 difference: 58; 95% CI: 13, 104).
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10 246 In analysis among HIV-infected patients with continuous vitamin D levels, each nmol/L
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12 247 higher vitamin D concentration was associated with a decrease of 3 CD8 and 3 CD3 T-
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14 248 cells per μL . Vitamin D insufficiency was associated with an average of 85 higher CD8
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16 249 T-cells/ μL during follow-up (95% CI: 4, 165). Similar results were observed when we
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18 250 restricted the analyses to the first eight months of follow-up, the duration of TB treatment
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20 251 at the time of the study in Tanzania. No relationship was observed with mean viral loads
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22 252 during follow-up in the patients who were HIV-infected at the time of enrollment.
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29 254 In analysis examining association of vitamin D status with nutritional parameters in the
30
31 255 entire period of follow-up, no significant relationship was observed with BMI, albumin,
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33 256 or hemoglobin concentrations (Table 5). During the first eight months of follow-up,
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35 257 patients with Vitamin D Insufficiency experienced a decline in BMI (Mean: -0.21 kg/m^2 ;
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37 258 95% CI: $-0.39, -0.02$), compared to patients with adequate vitamin D status. These results
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39 259 were more pronounced in HIV-uninfected patients (Mean: -0.34 ; 95% CI: $-0.60, -0.09$)
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41 260 and not significant in HIV-infected patients. HIV-infected patients with vitamin D
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43 261 insufficiency had increased albumin levels (Mean: 0.94 ; 95% CI: $0.55, 1.32$) during the
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45 262 first eight months of follow-up compared to patients with adequate vitamin D status.
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53 264 ***Discussion***
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3 265 In this study among 677 patients with tuberculosis in Tanzania, more than 61% of the
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5 266 participants had 25-hydroxyvitamin D concentrations below 75 nmol/L (75 nmol/L). 25-
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8 267 hydroxyvitamin D concentrations were associated with the season of blood draw, money
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10 268 spent on food per person per day, and height in HIV-uninfected participants and
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12 269 hemoglobin concentrations and CD4 T-cell counts among HIV-infected patients. Vitamin
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14 270 D insufficiency (<75 nmol/L) was not associated with mortality, HIV disease
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17 271 progression, or treatment failure during follow-up in the entire cohort. However, patients
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19 272 with vitamin D insufficiency had an increased risk of experiencing TB relapse during
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21 273 follow-up. Further, vitamin D insufficiency was associated with a decline in CD8 and
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23 274 CD3 T-cells in both the first eight months (the duration of TB treatment) and the entire
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25 275 period of follow-up. A similar relationship was observed with BMI in the first eight
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27 276 months of follow-up.
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34 278 Our study was conducted in Dar es Salaam, the largest urban center in Tanzania, and just
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36 279 six degrees south of the Equator. The prevalence of vitamin D insufficiency (>61%) in
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38 280 this study is higher than the approximately 40% found in a previous study among TB
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40 281 patients in Mwanza, Tanzania [12] and in our studies among HIV-infected pregnant
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42 282 women (~85% of them had stage 1 HIV disease, unlike this study) in Dar es Salaam [13
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44 283 14]. However, this prevalence is lower than what was observed in a cross-sectional study
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46 284 in South Africa, where 88% of HIV-uninfected and 97% of HIV-infected TB patients had
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48 285 vitamin D insufficiency. The mean vitamin D concentration in this study was 69.8
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50 286 nmol/L, compared to 86.5 nmol/L in the study in Mwanza and 28.8-40 nmol/L in the
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52 287 South African study. One study from Thailand observed similar levels (69.0 nmol/L) in
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3 288 TB patients [15]; Thailand is located at a similar distance from the Equator as Tanzania,
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5 289 though it is in the northern hemisphere. Similar to the study in South Africa, the vitamin
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7 290 D levels were lowest in our study in the dry winter season between July and October,
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9 291 though the differences were not as stark. For example, the mean vitamin D concentration
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11 292 in January to March in the South African study was 56.8 nmol/L and 30.8 nmol/L
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13 293 between July and September, whereas in our study, the concentrations were 74.8 nmol/L
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15 294 for January through February, and 66.3 nmol/L for July through October.
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22 296 Vitamin D is synthesized in the skin through the action of ultraviolet light on 7-
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24 297 dehydrocholesterol. Fatty fish, such as salmon and sardines, are good sources of vitamin
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26 298 D in the diet but are not widely available everywhere and are usually expensive.
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28 299 Increasing urbanization and a tendency to spend most time indoors are major factors that
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30 300 contribute to the inability of the skin to synthesize adequate amounts of vitamin D [16-
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32 301 18]. Additionally, the TB disease itself and/or the HIV co-infection in the participants in
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34 302 this study are probably the primary reasons for restricted physical activity, lack of
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36 303 adequate exposure to sunlight, and consequent low concentrations of vitamin D.
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43 305 Several other investigators have examined correlates of vitamin D status in TB patients.
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45 306 The study in Mwanza found that marital status, BMI, and serum transferrin receptor
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47 307 concentrations were correlated with vitamin D status. Though the first two were
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49 308 correlated with vitamin D status in our study in univariate analyses, neither remained
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51 309 significant in multivariate analyses. We didn't measure serum transferrin receptor in our
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53 310 study, though we did observe a correlation of vitamin D status with hemoglobin
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3 311 concentrations among the HIV-infected subset. Another study in South Africa found that
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5 312 TB status (active disease vs. latent infection), month of sampling, and BMI were
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8 313 significantly correlated with vitamin D status in multivariate analyses [3]. All patients in
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10 314 our study had active disease, and we didn't observe a relationship with BMI in our
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12 315 analyses. The study in South Africa incorporated only those correlates associated with
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14 316 serum 25(OH)D concentration with $P < 0.05$ in univariate analysis in the multivariate
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16 317 model. This may have precluded selection of important covariates and confounders, if
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18 318 measured, and produced biased estimates and confidence intervals; increasing the
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20 319 nominal significance level to 20% or more, as used in this study [19] can eliminate most
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22 320 of this bias. Most other studies have been with smaller sample sizes and have examined a
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24 321 limited set of covariates, compared to the current study.
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33 323 There was no association of vitamin D status with mortality or HIV disease progression
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35 324 in this cohort, unlike our previous studies among HIV-infected pregnant women [13 20]
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37 325 or HIV-infected adults [21 22] in Tanzania. The major difference is that in our earlier
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39 326 studies [13 20], a large majority (~85%) of the participants had stage 1 or asymptomatic
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41 327 HIV disease, whereas in this study, most of the individuals were already at stage 3
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43 328 disease.
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49 330 The association of vitamin D insufficiency with TB recurrence/relapse, primarily driven
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51 331 by the HIV-uninfected subset, is a novel finding in a longitudinal study and has important
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53 332 implications. Vitamin D deficiency has been linked to TB in several studies – a
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55 333 hypothesis perhaps initially generated by the observed seasonality of TB. *In vitro* and
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3 334 animal studies indicate that 1,25-dihydroxyvitamin D₃, the most active form of vitamin
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5 335 D, may increase mycobacterial killing by macrophages but also limits host damage by
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8 336 decreasing the gamma-interferon production [23-28]. In perhaps the strongest evidence to
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10 337 date for a role of vitamin D in tuberculosis, a study by Liu *et al* [29] found that the
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12 338 antimycobacterial response in humans is dependent on adequate availability of vitamin D.

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19 340 A few randomized trials of vitamin D supplementation in TB patients have been
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21 341 conducted in the past few years [4 5 30]. In a randomized trial that was conducted among
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23 342 365 TB patients in Guinea-Bissau starting antituberculosis treatment, overall mortality
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25 343 was 15% (54 of 365) at 1 year of follow-up and similar in both arms [5]. Martineau and
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27 344 colleagues didn't find a difference in median time to sputum culture conversion with
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29 345 vitamin D supplementation of 2.5 mg vitamin D₃ at enrollment, 14, 28, and 42 days after
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31 346 starting TB treatment in 126 adults with sputum smear-positive PTB [4]. A recent report
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33 347 by Coussens *et al* from a subset of the 126 adults included in the trial above stated that
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35 348 median time to sputum smear conversion in the intervention arm was significantly shorter
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37 349 than in the control arm (23 vs. 36 days; p=0.04) [30]. The lack of effect and concordance
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39 350 in most of these trials is probably due to the dose and dosing interval used. It is worth
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41 351 noting however, that large intermittent doses of vitamin D may result in
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43 352 supraphysiological concentrations in some cases, which may be more harmful than
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45 353 helpful in their effects on the immune system [31].
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3 355 Vitamin D insufficiency also was associated with T-cell subset counts only among the
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5 356 HIV-infected patients in this cohort. We can only speculate as to the reasons for the
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8 357 significantly higher increase in CD4 T-cells observed in patients with vitamin D
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10 358 insufficiency at baseline. One potential explanation is that HIV-infected patients with
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12 359 vitamin D insufficiency may experience more uncontrolled immune reconstitution,
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14 360 leading to a greater increase in CD4 T-cell counts, on treatment of TB, compared to
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16 361 patients with adequate vitamin D status. This may also explain why this relationship is
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18 362 observed only in the first eight months of follow-up and not subsequently.
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27 364 The results for CD8 and CD3 T-cells are consistent with our previous studies among
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29 365 HIV-infected women in Tanzania [13 14]. This could suggest a possible role of vitamin
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31 366 D in inflammation. Although, the conventional role of CD8 cells is as cytotoxic cells,
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33 367 they also are effector cells in inflammation [32]. The involvement of vitamin D in
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35 368 modulating CD8 cells is also indicated by the fact that CD8 cells express the highest
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37 369 concentration of vitamin D receptor of the immune cells [33]. Other studies also have
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39 370 found that vitamin D suppresses antigen-stimulated proinflammatory cytokine responses,
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41 371 which may help speed up resolution of inflammatory responses that can lead to increased
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43 372 risk of mortality among TB patients [30].
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50 374 TB, once known as 'consumption', is associated with significant wasting and weight loss.
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52 375 The observation that better vitamin D status among HIV-uninfected patients is associated
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54 376 with a greater increase in BMI during follow-up is likely related to decreased risk of
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3 377 relapse among these patients, as well as improvement in quality of life through
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5 378 mechanisms such as better metabolism that were not directly assessed in this study.
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8 379 The major strengths of this study include a large number of participants, more than half
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10 380 of whom were HIV-infected, comprehensive assessment of clinical, immunological,
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12 381 socio-demographic, and nutritional parameters, and a long duration of follow-up. On the
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14 382 other hand, the major limitation is the possibility of reverse causation and residual
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16 383 confounding. We have attempted to minimize this through rigorous analyses and
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18 384 adjusting for several potential confounders, including hemoglobin concentrations, HIV
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20 385 status, viral load, CD4 T-cells, and Karnofsky score, in most analyses. The study results
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22 386 are generalizable to most settings with a high TB burden and widely prevalent vitamin D
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24 387 insufficiency.
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389 In summary, our study results indicate that adequate vitamin D status is associated with
390 better clinical and nutritional parameters during follow-up in a cohort of TB patients in
391 Tanzania. While randomized trials of vitamin D supplementation among TB patients are
392 urgently warranted, it is also imperative to conduct dose-response studies to determine
393 ideal dose and duration for the supplement.

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23 405 U01AI045441 to support the study)
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28 407 The authors report NO conflict of interest
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33 409 *Author Contributions*
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35 410 SM wrote the first draft of the manuscript and analyzed and interpreted the data; FMM,
36
37 411 RJB, SA, WU, EV, and WWF were investigators of the parent trial and contributed to
38
39 412 field activities and oversight; RJB also helped with the analysis and interpretation of the
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41 413 data; all authors participated in study design and contributed to the final manuscript. All
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43 414 authors have also read and approved the final manuscript.
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References

1. WHO. Global Tuberculosis Control. Geneva: World Health Organization, 2011.
2. Chaisson RE, Martinson NA. Tuberculosis in Africa--combating an HIV-driven crisis. *N Engl J Med* 2008;**358**(11):1089-92 doi: 358/11/1089 [pii] 10.1056/NEJMp0800809[published Online First: Epub Date]].
3. Martineau AR, Nhamoyebonde S, Oni T, et al. Reciprocal seasonal variation in vitamin D status and tuberculosis notifications in Cape Town, South Africa. *Proc Natl Acad Sci U S A* 2011;**108**(47):19013-7 doi: 10.1073/pnas.1111825108[published Online First: Epub Date]].
4. Martineau AR, Timms PM, Bothamley GH, et al. High-dose vitamin D(3) during intensive-phase antimicrobial treatment of pulmonary tuberculosis: a double-blind randomised controlled trial. *Lancet* 2011;**377**(9761):242-50 doi: 10.1016/S0140-6736(10)61889-2[published Online First: Epub Date]].
5. Wejse C, Gomes VF, Rabna P, et al. Vitamin D as supplementary treatment for tuberculosis: a double-blind, randomized, placebo-controlled trial. *American Journal of Respiratory and Critical Care Medicine* 2009;**179**(9):843-50 doi: 10.1164/rccm.200804-567OC[published Online First: Epub Date]].
6. Villamor E, Mugusi F, Urassa W, et al. A Trial of the Effect of Micronutrient Supplementation on Treatment Outcome, T Cell Counts, Morbidity, and Mortality in Adults with Pulmonary Tuberculosis. *J Infect Dis* 2008;**197**(11):1499-505 doi: 10.1086/587846 10.1086/587846 [pii][published Online First: Epub Date]].

- 1
2
3 7. Karnofsky DA, Abelmann WH, Craver LF, et al. The use of nitrogen mustards in the
4
5 palliative treatment of cancer. *Cancer* 1948;**1**:634-56
6
7
- 8 8. Fawzi WW, Msamanga GI, Spiegelman D, et al. A randomized trial of multivitamin
9
10 supplements and HIV disease progression and mortality. *N Engl J Med* 2004;**351**(1):23-
11
12 32
13
- 14 9. Baum M, Cassetti L, Bonvehi P, et al. Inadequate dietary intake and altered nutrition
15
16 status in early HIV-1 infection. *Nutrition* 1994;**10**(1):16-20
17
18
- 19 10. Lohman TG, Roche AF, Martorell R. *Anthropometric standardization reference*
20
21 *manual*. Champaign, IL: Human Kinetics Books, 1988.
22
23
- 24 11. WHO. Interim proposal for a WHO staging system for HIV infection and disease.
25
26 *Wkly Epidemiol Rec* 1990;**65**:221-24
27
28
- 29 12. Friis H, Range N, Pedersen ML, et al. Hypovitaminosis D is common among
30
31 pulmonary tuberculosis patients in Tanzania but is not explained by the acute phase
32
33 response. *J Nutr* 2008;**138**(12):2474-80 doi: 138/12/2474 [pii]
34
35 10.3945/jn.108.094979[published Online First: Epub Date]].
36
37
- 38 13. Mehta S, Giovannucci E, Mugusi FM, et al. Vitamin D status of HIV-infected women
39
40 and its association with HIV disease progression, anemia, and mortality. *PLoS ONE*
41
42 2010;**5**(1):e8770 doi: 10.1371/journal.pone.0008770[published Online First: Epub Date]].
43
44
- 45 14. Mehta S, Spiegelman D, Aboud S, et al. Lipid-soluble vitamins A, D, and E in HIV-
46
47 infected pregnant women in Tanzania. *European journal of clinical nutrition*
48
49 2010;**64**(8):808-17 doi: 10.1038/ejcn.2010.76[published Online First: Epub Date]].
50
51
- 52 15. Davies PD, Church HA, Bovornkitti S, et al. Altered vitamin D homeostasis in
53
54 tuberculosis. *Intern Med (Thailand)* 1988;**4**:45-47
55
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16. Norman AW. Nutritional Aspects of Vitamin D. <http://vitamind.ucr.edu/nutri.html>. Accessed Oct 9, 2006. 1999. <http://vitamind.ucr.edu/nutri.html> (accessed Oct 9, 2006).
17. Utiger RD. The need for more vitamin D. *N Engl J Med* 1998;**338**(12):828-9
18. Holick MF. High prevalence of vitamin D inadequacy and implications for health. *Mayo Clin Proc* 2006;**81**(3):353-73
19. Greenland S. Modeling and variable selection in epidemiologic analysis. *Am J Public Health* 1989;**79**(3):340-9
20. Mehta S, Mugusi FM, Spiegelman D, et al. Vitamin D status and its association with morbidity including wasting and opportunistic illnesses in HIV-infected women in Tanzania. *AIDS Patient Care STDS* 2011;**25**(10):579-85 doi: 10.1089/apc.2011.0182[published Online First: Epub Date].
21. Sudfeld CR, Giovannucci EL, Isanaka S, et al. Vitamin D Status and Incidence of Pulmonary Tuberculosis, Opportunistic Infections, and Wasting Among HIV-Infected Tanzanian Adults Initiating Antiretroviral Therapy. *The Journal of infectious diseases* 2013;**207**(3):378-85 doi: 10.1093/infdis/jis693[published Online First: Epub Date].
22. Sudfeld CR, Wang M, Aboud S, et al. Vitamin D and HIV progression among Tanzanian adults initiating antiretroviral therapy. *PloS one* 2012;**7**(6):e40036 doi: 10.1371/journal.pone.0040036[published Online First: Epub Date].
23. Thoma-Uszynski S, Stenger S, Takeuchi O, et al. Induction of direct antimicrobial activity through mammalian toll-like receptors. *Science* 2001;**291**(5508):1544-7
24. Waters WR, Palmer MV, Nonnecke BJ, et al. Mycobacterium bovis infection of vitamin D-deficient NOS2^{-/-} mice. *Microb Pathog* 2004;**36**(1):11-7

- 1
2
3 25. Waters WR, Nonnecke BJ, Foote MR, et al. Mycobacterium bovis bacille Calmette-
4 Guerin vaccination of cattle: activation of bovine CD4+ and gamma delta TCR+ cells and
5
6 modulation by 1,25-dihydroxyvitamin D3. Tuberculosis (Edinb) 2003;**83**(5):287-97
7
8
9
10 26. Waters WR, Nonnecke BJ, Rahner TE, et al. Modulation of Mycobacterium bovis-
11 specific responses of bovine peripheral blood mononuclear cells by 1,25-
12
13 dihydroxyvitamin D(3). Clin Diagn Lab Immunol 2001;**8**(6):1204-12
14
15
16
17 27. Kawakami K, Teruya K, Tohyama M, et al. [A therapeutic trial of experimental
18 tuberculosis with gamma-interferon in an immunocompromised mouse model]. Kekkaku
19
20 1994;**69**(10):607-13
21
22
23
24 28. McMurray DN, Bartow RA, Mintzer CL, et al. Micronutrient status and immune
25 function in tuberculosis. Ann N Y Acad Sci 1990;**587**:59-69
26
27
28
29 29. Liu PT, Stenger S, Li H, et al. Toll-Like Receptor Triggering of a Vitamin D-
30 Mediated Human Antimicrobial Response. Science 2006:1123933 doi:
31
32 10.1126/science.1123933[published Online First: Epub Date]].
33
34
35
36 30. Coussens AK, Wilkinson RJ, Hanifa Y, et al. Vitamin D accelerates resolution of
37 inflammatory responses during tuberculosis treatment. Proc Natl Acad Sci 2012:Epub
38
39
40 31. Martineau AR. Bolus-dose vitamin D and prevention of childhood pneumonia. Lancet
41 2012;**379**(9824):1373-5 doi: 10.1016/S0140-6736(12)60405-X[published Online First:
42
43 Epub Date]].
44
45
46
47 32. Babbe H, Roers A, Waisman A, et al. Clonal expansions of CD8(+) T cells dominate
48 the T cell infiltrate in active multiple sclerosis lesions as shown by micromanipulation
49 and single cell polymerase chain reaction. J Exp Med 2000;**192**(3):393-404
50
51
52
53
54
55
56
57
58
59
60

1
2
3 33. Veldman CM, Cantorna MT, DeLuca HF. Expression of 1,25-dihydroxyvitamin D(3)
4 receptor in the immune system. Arch Biochem Biophys 2000;374(2):334-8 doi:
5
6

7
8 10.1006/abbi.1999.1605
9

10 S0003-9861(99)91605-3 [pii][published Online First: Epub Date].
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Figure Legends

1. Distribution of 25-hydroxyvitamin D concentrations at baseline (nmol/L)
2. Distribution of 25-hydroxyvitamin D concentrations by season of blood draw;
Season 1: Dry (January-February); Season 2: Long Rains (March-June); Season 3:
Dry (July-October); Season 4: Short Rains (November-December)

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Tables

Table 1	Baseline Characteristics of the Study Population (n=677)		
		HIV-infected (n=344)	HIV-uninfected (n=333)
Variable		Mean (Standard Deviation)	Mean (Standard Deviation)
Age, years		34.4 ± 8.6	30.2 ± 9.2
Money spent on food per person per day, Tanzanian Shillings*		587.3 ± 445.9	580.1 ± 684.2
Hemoglobin, g/dL		9.9 ± 1.8	11.1 ± 1.7
Albumin, g/dL		2.8 ± 1.0	3.2 ± 1.1
CD3 T-cell count, cells/μL		1228.0 ± 608.5	1195.9 ± 404.8
CD4 T-cell counts, cells/μL		327.2 ± 246.2	709.2 ± 250.8
CD8 T-cell counts, cells/μL		826.9 ± 447.5	427.5 ± 188.2
Log(10) Viral Load, copies/mL		4.6 ± 1.0	N/A
Body Mass Index, kg/m ²		19.4 ± 2.8	18.8 ± 2.5
Mid-Upper Arm Circumference, cm		23.4 ± 2.7	23.1 ± 2.7
Follow-up time, days		916.8 ± 507.4	1532.9 ± 331.4
		n (%)	n (%)
Vitamin D insufficiency (serum 25- hydroxyvitamin D <75 nmol/L)		218 (63.4%)	200 (60.1%)

	Vitamin D deficiency (serum 25-hydroxyvitamin D <50 nmol/L)	55 (16.0%)	51 (15.3%)
	Sex		
	Male	203 (59.0%)	257 (77.2%)
	Female	141 (41.0%)	76 (22.8%)
	Center		
	Mwananyamala	79 (23.0%)	88 (26.4%)
	Temeke	102 (29.7%)	83 (24.9%)
	Tandale	83 (24.1%)	91 (27.3%)
	Mbgala	31 (9.0%)	70 (21.0%)
	Amana	49 (14.2%)	1 (.3%)
	Karnofsky Score <70%	45 (13.1%)	29 (8.7%)
	Education Group		
	None	29 (8.4%)	36 (10.8%)
	Low <5years	35 (10.2%)	31 (9.3%)
	Primary 5-8 years	238 (69.2%)	233 (70.0%)
	Secondary/University	42 (12.2%)	33 (9.9%)

Cohabits with a Partner		200 (58.1%)	168 (50.5%)
Assets at home			
	None	92 (26.9%)	108 (32.4%)
	One	89 (26.0%)	85 (25.5%)
	2-3	122 (35.7%)	114 (34.2%)
	4-5	39 (11.4%)	26 (7.8%)
WHO HIV Disease Stage			
	3	240 (90.9%)	N/A
	4	24 (9.1%)	
CD4 T-cell categories, cells/ μ L			
	0-199	97 (35.9%)	0 (.0%)
	200-499	116 (43.0%)	69 (22.9%)
	500+	57 (21.1%)	232 (77.1%)
WHO BMI Group, kg/m ²			
	<16	26 (7.7%)	33 (9.9%)
	16-16.99	37 (10.9%)	45 (13.6%)
	17-18.49	73 (21.5%)	88 (26.5%)
	18.5-19.99	79 (23.3%)	70 (21.1%)
	20-21.99	77 (22.7%)	69 (20.8%)

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	22+	47 (13.9%)	27 (8.1%)
* 1 US Dollar \cong 1000 Tanzanian Shillings at the time of the study			

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Table 2		Vitamin D Status and Mortality and HIV Disease Progression in HIV-infected TB Patients			
		Univariate		Multivariate	
Outcome	n/N (%)	RR (95% CI)	p-value	RR (95% CI)	p-value
Mortality					
Vitamin D insufficiency (<75 nmol/L)	61/218 (28.0%)	0.73 (0.50, 1.08)	0.12	0.70 (0.47, 1.04)	0.08
Adequate Vitamin D	43/126 (34.1%)				
Vitamin D deficient (<50 nmol/L)	20/55 (36.4%)	1.34 (0.82, 2.18)	0.25	0.91 (0.55 1.50)	0.71
Not deficient	84/289 (29.1%)				
Continuous Vitamin D (nmol/L)		1.00 (0.99, 1.01)	0.49	1.01 (1.00, 1.02)	0.15
HIV Disease Progression					

Vitamin D insufficiency (<75 nmol/L)	46/150 (30.7%)	1.10 (0.67, 1.82)	0.71	1.08 (0.64, 1.82)	0.78
Adequate Vitamin D	23/90 (25.6%)	Reference		Reference	
Vitamin D deficient (<50 nmol/L)	14/34 (41.2%)	1.91 (1.05, 3.44)	0.03	1.48 (0.78, 2.82)	0.23
Not deficient	55/206 (26.7%)	Reference		Reference	
Continuous Vitamin D (nmol/L)		0.99 (0.98, 1.01)	0.30	1.00 (0.99, 1.01)	0.57
p-values obtained using Cox Proportional Hazards Regression; RR: Risk Ratio; 95% CI: 95% Confidence Interval					
Multivariate analyses adjusted for Age, Karnofsky Score, Baseline Hemoglobin, Viral Load, HIV Status, CD4 Counts, and Micronutrient Supplementation					

Table 3		Vitamin D Status and Treatment Outcomes in TB Patients				
			Univariate		Multivariate	
<i>Outcome</i>	<i>n/N (%)</i>		<i>RR (95% CI)</i>	<i>p-value</i>	<i>RR (95% CI)</i>	<i>p-value</i>
Treatment Failure by 1 month post-treatment initiation						
Vitamin D insufficiency (<75 nmol/L)	58/298 (19.5%)		1.06 (0.72, 1.55)	0.77	1.02 (0.70, 1.49)	0.93
Adequate Vitamin D	34/185 (18.4%)					
Vitamin D deficient (<50 nmol/L)	15/75 (20.0%)		1.06 (0.65, 1.74)	0.82	1.13 (0.69, 1.86)	0.63
Not deficient	77/408 (18.9%)					
Continuous Vitamin D (nmol/L)			1.00 (0.99, 1.01)	0.49	1.00 (0.99, 1.01)	0.50
Any Relapse (relapse after 1 month post-treatment initiation if culture negative at 1 month)						
Vitamin D insufficiency	51/227 (22.5%)		1.56 (0.98, 2.48)	0.06	1.66 (1.04, 2.64)	0.03

	(<75 nmol/L)						
	Adequate Vitamin D	21/146 (14.4%)					
	Vitamin D deficient (<50 nmol/L)	13/56 (23.2%)	1.25 (0.73, 2.12)	0.41	1.40 (0.82, 2.39)	0.21	
	Not deficient	59/317 (18.6%)					
	Continuous Vitamin D (nmol/L)		0.99 (0.98, 1.00)	0.06	0.99 (0.98, 1.00)	0.04	
p-values obtained using Binomial Regression; RR: Risk Ratio; 95% CI: 95% Confidence Interval							
Multivariate analyses adjusted for Age, Karnofsky Score, Baseline Hemoglobin, Viral Load, HIV Status, CD4 Counts, and Micronutrient Supplementation							

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Table 4	Vitamin D Status and T-cell Counts (cells/ μ L) in TB Patients													
	CD4 T-cells					CD8 T-cells					CD3 T-cells			
Outcome	Adequate vitamin D, mean difference (SD) ^a	Vitamin D insufficiency, mean difference (95% CI) ^b	Vitamin D insufficiency, adjusted mean difference (95% CI) ^c	p-value		Adequate vitamin D, mean difference (SD) ^a	Vitamin D insufficiency, mean difference (95% CI) ^b	Vitamin D insufficiency, adjusted mean difference (95% CI) ^c	p-value		Adequate vitamin D, mean (SD) ^a	Vitamin D insufficiency, mean difference (95% CI) ^b	Vitamin D insufficiency, adjusted mean difference (95% CI) ^c	p-value

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Entire follow-up: HIV-infected patients													
Vitamin D insufficiency (<75 nmol/L)	300 (234)	17 (-23, 56)	21 (-18, 59)	0.2 9	902 (457)	88 (7, 169)	85 (4, 165)	0.0 4	1298 (635)	101 (-4, 206)	103 (-5, 212)	0.0 6	
Vitamin D deficient (<50 nmol/L)	333 (225)	21 (-34, 76)	30 (-26, 86)	0.2 9	957 (424)	105 (-9, 219)	114 (-6, 234)	0.0 6	1392 (595)	104 (-47, 255)	125 (-28, 279)	0.1 1	
Continuous Vitamin D (per nmol/L)		0 (-1, 1)	-1 (-1, 0)	0.2 6		-3 (-5, -1)	-3 (-5, -1)	0.0 04		-3 (-5, -1)	-3 (-6, -1)	0.0 1	
Entire follow-up: HIV-uninfected patients													
Vitamin D	771	-2 (-)	3 (-45, 3)	0.9	508	-25 (-)	-22 (-)	0.2	1351	-37 (-)	-28 (-)	0.4	

	insufficiency (<75 nmol/L)	(235)	49, 45)	51)	1	(209)	63, 14)	60, 17)	7	(400)	109, 35)	99, 44)	5
	Vitamin D deficient (<50 nmol/L)	781 (241)	-34 (-99, 30)	-34 (-101, 32)	0.3 1	500 (195)	-1 (-64, 62)	3 (-61, 67)	0.9 3	1354 (397)	-33 (-136, 71)	-28 (-134, 79)	0.6 1
	Continuous Vitamin D (per nmol/L)		0 (-1, 1)	0 (-1, 1)	0.9 7		0 (-1, 1)	0 (-1, 1)	0.8 3		0 (-1, 2)	0 (-2, 2)	0.9 0
First 8 months of follow-up: HIV-infected patients													
	Vitamin D insufficiency (<75 nmol/L)	316 (237)	54 (8, 100)	58 (13, 104)	0.0 1	868 (470)	132 (29, 235)	119 (15, 223)	0.0 2	1279 (670)	190 (42, 337)	179 (28, 331)	0.0 2

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	Vitamin D deficient (<50 nmol/L)	372 (264)	36 (-25, 97)	41 (-20, 101)	0.1 9	963 (471)	63 (-77, 203)	75 (-72, 221)	0.3 2	1443 (689)	67 (-125, 259)	101 (-93, 295)	0.3 1
	Continuous Vitamin D (per nmol/L)		-1 (-2, 0)	-1 (-2, 0)	0.0 1		-4 (-6, -1)	-4 (-6, -1)	0.0 02		-4 (-7, -1)	-5 (-8, -2)	0.0 03
First 8 months of follow-up: HIV-uninfected patients													
	Vitamin D insufficiency (<75 nmol/L)	724 (243)	1 (-52, 53)	6 (-47, 59)	0.8 2	461 (232)	-22 (-63, 20)	-17 (-57, 22)	0.3 9	1248 (446)	-38 (-121, 46)	-27 (-106, 52)	0.5 0
	Vitamin D deficient	731	-7 (-)	-7 (-)	0.8	454	4 (-73,)	5 (-71,)	0.9	1247	14 (-)	17 (-)	0.8

	(<50 nmol/L)	(237)	95, 80)	96, 81)	7	(209)	81)	81)	0	(410)	124, 153)	123, 156)	2
	Continuous Vitamin D (per nmol/L)		0 (-2,1)	0 (-2,1)	0.5 4		0 (-1, 1)	0 (-1, 1)	0.7 3		0 (-2, 1)	0 (-2, 1)	0.5 7

^a Data are the means (SD) of the average measurement during follow-up for each participant

^b Data are the mean difference between the low and the adequate vitamin D group, as defined in Column B. The mean differences, 95% confidence intervals (CIs), and corresponding p-values were estimated from generalized estimating equations, after adjustment for baseline measurements, follow-up time, and treatment (micronutrients vs. placebo) group.

^c Multivariate analyses additionally adjusted for Age, Karnofsky Score, and Baseline Hemoglobin

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Table 5 Vitamin D Status and Nutritional Parameters in TB Patients												
	Body Mass Index (kg/m ²)				Albumin concentration (g/dL)				Hemoglobin concentration (g/dL)			
Outcome	Adequate vitamin D, mean (SD) ^a	Vitamin D insufficiency, mean difference (95% CI) ^b	Vitamin D insufficiency, adjusted mean difference (95% CI) ^c	p-value	Adequate vitamin D, mean (SD) ^a	Vitamin D insufficiency, mean difference (95% CI) ^b	Vitamin D insufficiency, adjusted mean difference (95% CI) ^c	p-value	Adequate vitamin D, mean (SD) ^a	Vitamin D insufficiency, mean difference (95% CI) ^b	Vitamin D insufficiency, adjusted mean difference (95% CI) ^c	p-value
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follow-up:													
All patients													
Vitamin D insufficiency (<75 nmol/L)	21.20 (2.80)	-0.06 (-0.30, 0.17)	-0.08 (-0.30, 0.14)	0.46	3.42 (0.74)	-0.05 (-0.14, 0.04)	0.00 (-0.08, 0.08)	0.9 7	12.65 (1.80)	-0.16 (-0.40, 0.08)	-0.18 (-0.41, 0.05)	0. 12	
Vitamin D deficient (<50 nmol/L)	21.23 (3.00)	-0.16 (-0.46, 0.14)	-0.14 (-0.44, 0.15)	0.34	3.42 (0.72)	-0.05 (-0.17, 0.07)	0.02 (-0.08, 0.13)	0.65	12.42 (1.87)	0.15 (-0.16, 0.45)	0.17 (-0.11, 0.45)	0.2 4	

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	Continuous Vitamin D (per nmol/L)	0.00 (0.00, 0.01)	0.00 (0.00, 0.01)	0.30			0.002 (0.00, 0.004)	0.000 (-0.002, 0.002)	0.90		0.00 (-0.01, 0.01)	0.00 (0.00, 0.01)	0.8 5	
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	First 8 months of follow-up: All patients													
	Vitamin D insufficiency (<75 nmol/L)	20.96 (2.73)	-0.20 (-0.40, -0.01)	-0.21 (-0.39, -0.02)	0.03		3.42 (1.09)	-0.01 (-0.18, 0.16)	0.04 (-0.13, 0.21)	0.65	12.12 (1.85)	-0.01 (-0.28, 0.26)	-0.04 (-0.31, 0.23)	0.7 8

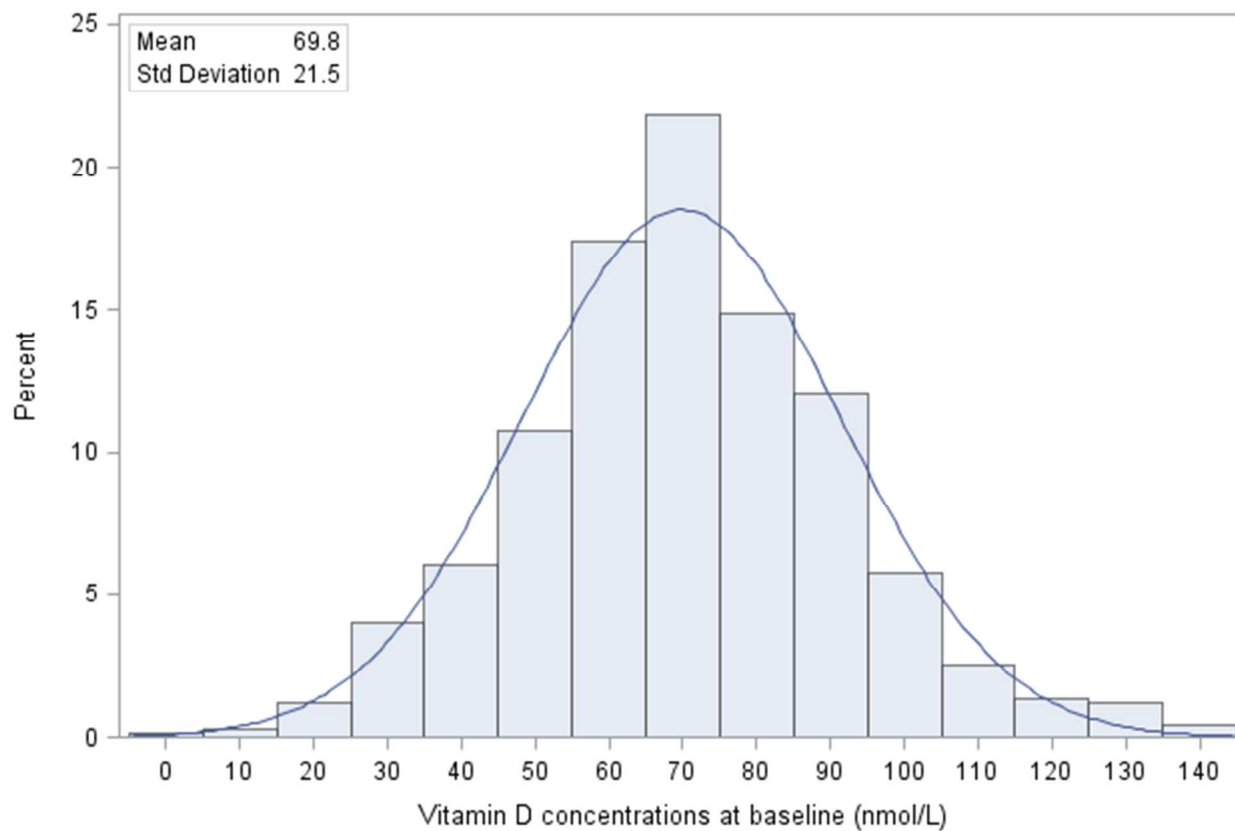
Vitamin D deficient (<50 nmol/L)	20.85 (2.84)	0.00 (-0.25, 0.25)	0.04 (-0.21, 0.29)	0.78	3.41 (1.08)	-0.11 (-0.32, 0.10)	-0.05 (-0.27, 0.17)	0.64		11.92 (1.99)	0.16 (-0.18, 0.50)	0.21 (-0.10, 0.53)	0.1 9
Continuous Vitamin D (per nmol/L)		0.00 (0.00, 0.01)	0.00 (0.00, 0.01)	0.38		0.000 (-0.003, 0.005)	0.000 (-0.004, 0.004)	0.87			0.00 (-0.01, 0.00)	0.00 (-0.01, 0.00)	0.5 7

^aData are the means (SD) of the average measurement during follow-up for each participant

^bData are the mean difference between the low and the adequate vitamin D group, as defined in Column B. The mean differences, 95% confidence intervals (CIs), and corresponding p-values were estimated from generalized estimating equations, after adjustment for baseline

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5 measurements, follow-up time, and treatment (micronutrients vs. placebo) group.
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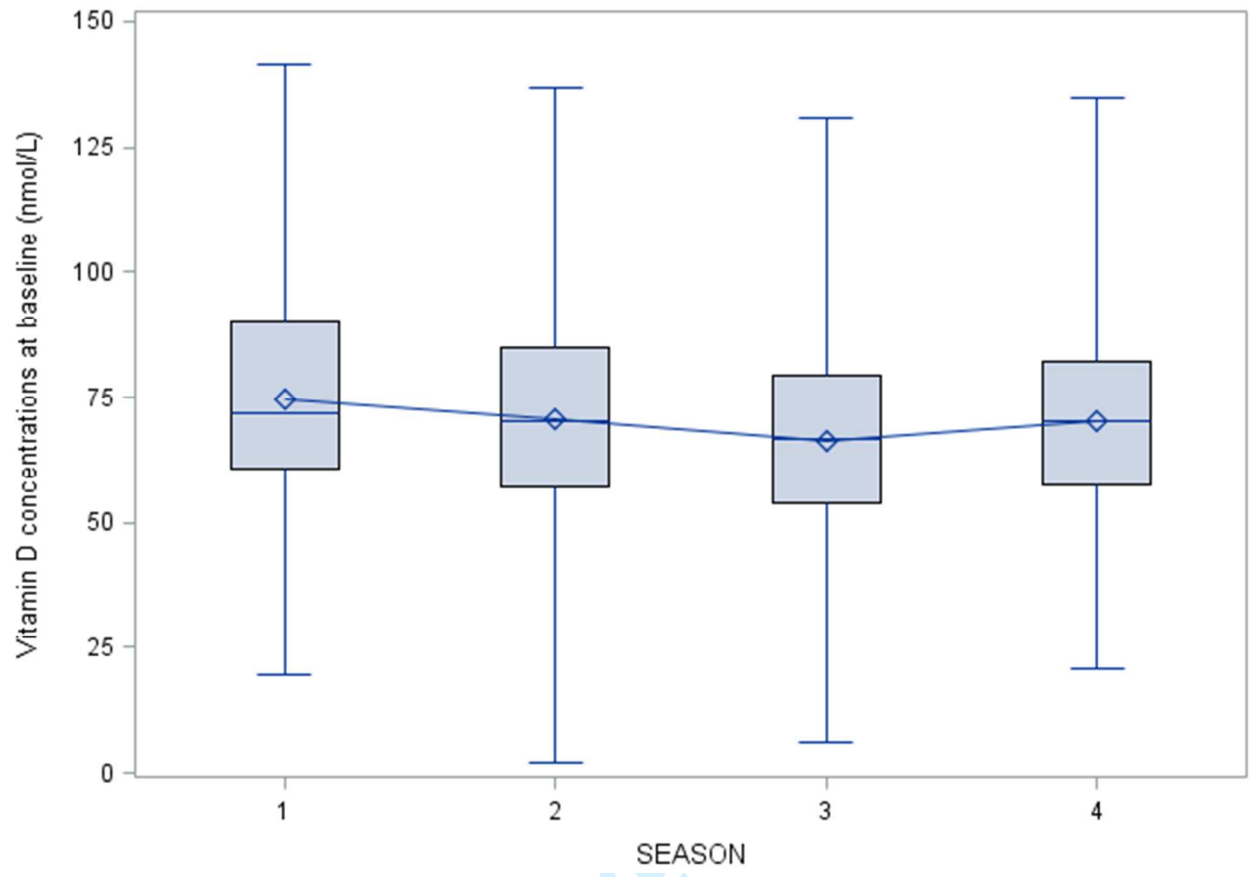
7 ^c Multivariate analyses additionally adjusted for Age, Karnofsky Score, Baseline Hemoglobin, Viral Load, CD4 Count, and HIV Status;
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9 HIV status removed from the model where the results are stratified by HIV status. Viral Load also removed from the model in HIV-
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11 uninfected individuals.
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Supplemental Table 1		Correlates of Vitamin D insufficiency (25(OH)D < 75 nmol/L) in HIV-uninfected TB patients			
		Univariate (p<0.20)		Multivariate (p<0.05)	
Variable		RR (95% CI)	p-value	RR (95% CI)	p-value
Season			0.002		0.002
	1: Dry (Jan-Feb)	Ref.		Ref.	
	2: Long Rains (Mar-Jun)	1.07 (0.74, 1.53)		1.09 (0.76, 1.56)	
	3: Dry (Jul-Oct)	1.50 (1.07, 2.09)		1.50 (1.08, 2.08)	
	4: Short Rains (Nov-Dec)	1.29 (0.84, 1.96)		1.26 (0.84, 1.90)	
Sex					
	Female	1.19 (0.99, 1.43)	0.07		
Cohabits with a partner		0.89 (0.75, 1.06)	0.19		
Money spent per person per day on food, per 1000 Tanzanian Shillings*		0.76 (0.59, 0.98)	0.04	0.76 (0.59, 0.98)	0.03
Money spent per person per day on food quartiles			0.14		
	0:<250	1.36 (1.03, 1.80)			
	1:250-499	1.11 (0.82, 1.52)			
	2:500-750	1.13 (0.84, 1.53)			
	3:>750	Ref.			
AFB Culture positive at baseline		1.17 (0.94, 1.46)	0.16		
Number of colonies in AFB culture			0.16		
	1	Ref.			
	2	1.01 (0.66, 1.56)			
	3	0.95 (0.63, 1.44)			
	4	1.05 (0.70, 1.58)			

	5	1.27 (1.01, 1.59)			
	Received TB treatment in the past 5 years	1.41 (0.97, 2.04)	0.07		
	Hemoglobin, g/dL	0.92 (0.87, 0.97)	0.004		
	CD4 T-cells, 100 cells/ μ L	1.03 (0.99, 1.07)	0.12		
	CD3 T-cells, 100 cells/ μ L	1.02 (1.00, 1.04)	0.12		
	Depressed >2 weeks, ever	1.17 (0.97, 1.42)	0.10		
	Dysentery	0.23 (0.04, 1.41)	0.11		
	Outpatient visit	1.14 (0.95, 1.38)	0.16		
	Skin rash	0.73 (0.46, 1.16)	0.18		
	Height quartiles, cm		0.01		0.01
	<158.1	Ref.		Ref.	
	158.1-164.0	1.08 (0.87, 1.32)		1.12 (0.93, 1.36)	
	164.1-169.5	0.76 (0.59, 0.98)		0.82 (0.64, 1.04)	
	169.6+	0.77 (0.60, 0.99)		0.81 (0.64, 1.03)	
	Weight, kg	0.99 (0.97, 1.00)	0.03		
	WHO BMI groups, kg/m ²		0.14		
	<16	1.14 (0.88, 1.48)			
	16-16.99	0.98 (0.74, 1.29)			
	17-18.49	Ref.			
	18.5-19.99	0.76 (0.57, 1.02)			
	20-21.99	0.89 (0.68, 1.15)			
	22+	1.11 (0.83, 1.48)			
	Mid-Upper Arm Circumference (MUAC) <22 cm	1.27 (1.07, 1.51)	0.01		
	Triceps Skinfold Thickness,	1.02 (1.00, 1.03)	0.09		

cm							
p-values obtained using Binomial Regression; RR: Risk Ratio; 95% CI: 95% Confidence Interval; * 1 US Dollar \cong 1000 Tanzanian Shillings at the time of the study							

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Supplemental Table 2		Correlates of Vitamin D insufficiency (serum 25(OH)D <75 nmol/L) in HIV-infected TB patients			
		Univariate (p<0.20)		Multivariate (p<0.05)	
Variable		RR (95% CI)	P-value	RR (95% CI)	P-value
Age		0.99 (0.98, 1.00)	0.01		
Center			0.03		
	Mwananyamala	1.05 (0.86, 1.27)			
	Temeke	Ref.			
	Tandale	0.80 (0.63, 1.02)			
	Mbgala	0.67 (0.44, 1.01)			
	Amana	1.03 (0.82, 1.29)			
Cohabits with a partner		0.90 (0.77, 1.05)	0.19		
Assets at home			0.07		
	0:none	1.36 (1.03, 1.80)			
	1:one	0.88 (0.66, 1.16)			
	2:2-3	0.87 (0.67, 1.14)			
	3:4-5	Ref.			
Received TB treatment in the past 5 years		0.58 (0.31, 1.10)	0.10		
Hemoglobin, g/dL		0.95 (0.90, 1.00)	0.04	0.93 (0.89, 0.98)	0.007
Albumin, U/L		0.93 (0.86, 1.02)	0.13		
CD4 T-cells, 100 cells/ μ L		1.02 (0.99, 1.05)	0.12	1.04 (1.01, 1.07)	0.02
Depressed >2 weeks, ever		0.80 (0.61, 1.05)	0.11		
Hospitalization		1.30 (0.94, 1.80)	0.11		
Skin rash		1.32 (1.07, 1.64)	0.01		
Extrapulmonary TB		1.35 (0.93, 1.96)	0.11		

Online Supporting Material

Height, cm		0.99 (0.98, 1.00)	0.08		
Weight, kg		0.99 (0.98, 1.00)	0.11		
WHO Body Mass Index (BMI) groups, kg/m ²			0.06		
	<16	0.81 (0.57, 1.16)			
	16-16.99	0.99 (0.76, 1.27)			
	17-18.49	Ref.			
	18.5-19.99	0.80 (0.63, 1.02)			
	20-21.99	0.75 (0.58, 0.96)			
	22+	1.05 (0.84, 1.30)			
Mid-Upper Arm Circumference (MUAC) quartiles, cm			0.02		
	<=21	Ref.			
	21.1-23.0	1.09 (0.90, 1.32)			
	23.1-24.9	0.81 (0.61, 1.08)			
	25+	0.82 (0.66, 1.03)			
p-values obtained using Binomial Regression; RR: Risk Ratio; 95% CI: 95% Confidence Interval					

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

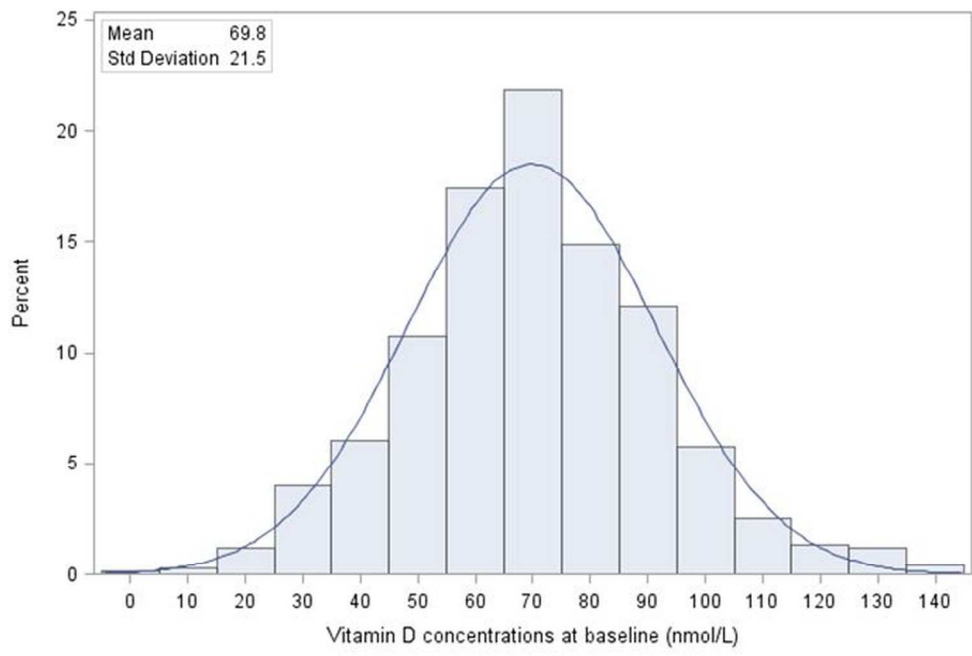
Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	2
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	2-3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5
Objectives	3	State specific objectives, including any prespecified hypotheses	6
Methods			
Study design	4	Present key elements of study design early in the paper	6-8
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6-8
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	6-8
		(b) For matched studies, give matching criteria and number of exposed and unexposed	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6, 9
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	9-10
Bias	9	Describe any efforts to address potential sources of bias	10
Study size	10	Explain how the study size was arrived at	10
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	10
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	9-10
		(b) Describe any methods used to examine subgroups and interactions	9-10
		(c) Explain how missing data were addressed	9-10
		(d) If applicable, explain how loss to follow-up was addressed	9-10
		(e) Describe any sensitivity analyses	NA
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	10, Tables
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	10-11
		(b) Indicate number of participants with missing data for each variable of interest	10
		(c) Summarise follow-up time (eg, average and total amount)	11
Outcome data	15*	Report numbers of outcome events or summary measures over time	Tables
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Tables
		(b) Report category boundaries when continuous variables were categorized	Tables
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	10-13
Discussion			
Key results	18	Summarise key results with reference to study objectives	13
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	14-19
Generalisability	21	Discuss the generalisability (external validity) of the study results	18-19
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	20

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.

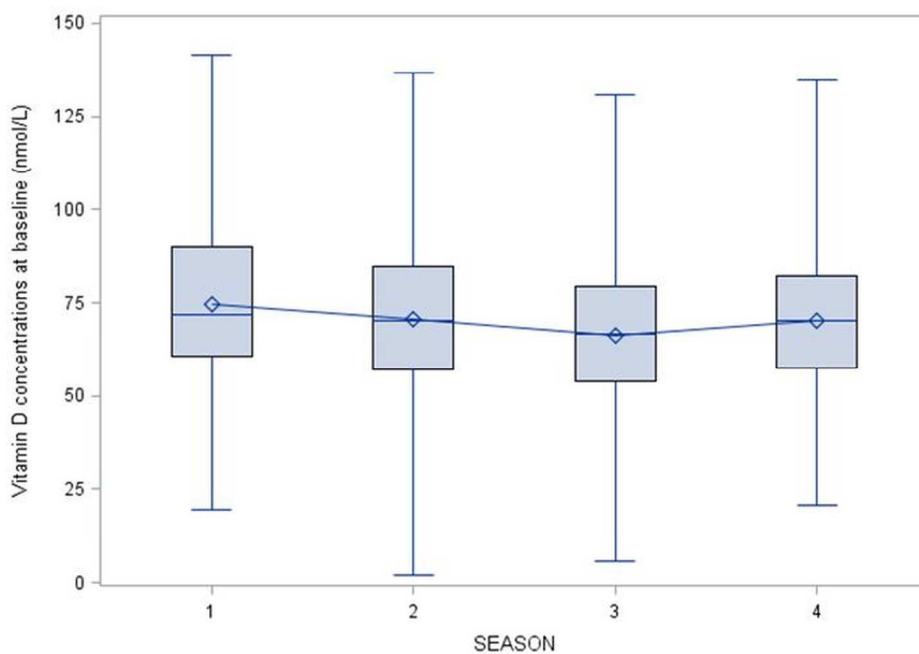
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Supplemental Table 1		Correlates of Vitamin D insufficiency (25(OH)D < 75 nmol/L) in HIV-uninfected TB patients			
		Univariate (p<0.20)		Multivariate (p<0.05)	
Variable		RR (95% CI)	p-value	RR (95% CI)	p-value
Season			0.002		0.002
	1: Dry (Jan-Feb)	Ref.		Ref.	
	2: Long Rains (Mar-Jun)	1.07 (0.74, 1.53)		1.09 (0.76, 1.56)	
	3: Dry (Jul-Oct)	1.50 (1.07, 2.09)		1.50 (1.08, 2.08)	
	4: Short Rains (Nov-Dec)	1.29 (0.84, 1.96)		1.26 (0.84, 1.90)	
Sex					
	Female	1.19 (0.99, 1.43)	0.07		
Cohabits with a partner		0.89 (0.75, 1.06)	0.19		
Money spent per person per day on food, per 1000 Tanzanian Shillings*		0.76 (0.59, 0.98)	0.04	0.76 (0.59, 0.98)	0.03
Money spent per person per day on food quartiles			0.14		
	0:<250	1.36 (1.03, 1.80)			
	1:250-499	1.11 (0.82, 1.52)			
	2:500-750	1.13 (0.84, 1.53)			
	3:>750	Ref.			
AFB Culture positive at baseline		1.17 (0.94, 1.46)	0.16		
Number of colonies in AFB culture			0.16		
	1	Ref.			
	2	1.01 (0.66, 1.56)			
	3	0.95 (0.63, 1.44)			
	4	1.05 (0.70, 1.58)			

	5	1.27 (1.01, 1.59)			
	Received TB treatment in the past 5 years	1.41 (0.97, 2.04)	0.07		
	Hemoglobin, g/dL	0.92 (0.87, 0.97)	0.004		
	CD4 T-cells, 100 cells/ μ L	1.03 (0.99, 1.07)	0.12		
	CD3 T-cells, 100 cells/ μ L	1.02 (1.00, 1.04)	0.12		
	Depressed >2 weeks, ever	1.17 (0.97, 1.42)	0.10		
	Dysentery	0.23 (0.04, 1.41)	0.11		
	Outpatient visit	1.14 (0.95, 1.38)	0.16		
	Skin rash	0.73 (0.46, 1.16)	0.18		
	Height quartiles, cm		0.01		0.01
	<158.1	Ref.		Ref.	
	158.1-164.0	1.08 (0.87, 1.32)		1.12 (0.93, 1.36)	
	164.1-169.5	0.76 (0.59, 0.98)		0.82 (0.64, 1.04)	
	169.6+	0.77 (0.60, 0.99)		0.81 (0.64, 1.03)	
	Weight, kg	0.99 (0.97, 1.00)	0.03		
	WHO BMI groups, kg/m ²		0.14		
	<16	1.14 (0.88, 1.48)			
	16-16.99	0.98 (0.74, 1.29)			
	17-18.49	Ref.			
	18.5-19.99	0.76 (0.57, 1.02)			
	20-21.99	0.89 (0.68, 1.15)			
	22+	1.11 (0.83, 1.48)			
	Mid-Upper Arm Circumference (MUAC) <22 cm	1.27 (1.07, 1.51)	0.01		
	Triceps Skinfold Thickness,	1.02 (1.00, 1.03)	0.09		

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cm						
p-values obtained using Binomial Regression; RR: Risk Ratio; 95% CI: 95% Confidence Interval; * 1 US Dollar \cong 1000 Tanzanian Shillings at the time of the study						

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Supplemental Table 2		Correlates of Vitamin D insufficiency (serum 25(OH)D <75 nmol/L) in HIV-infected TB patients			
		Univariate (p<0.20)		Multivariate (p<0.05)	
Variable		RR (95% CI)	P-value	RR (95% CI)	P-value
Age		0.99 (0.98, 1.00)	0.01		
Center			0.03		
	Mwananyamala	1.05 (0.86, 1.27)			
	Temeke	Ref.			
	Tandale	0.80 (0.63, 1.02)			
	Mbgala	0.67 (0.44, 1.01)			
	Amana	1.03 (0.82, 1.29)			
Cohabits with a partner		0.90 (0.77, 1.05)	0.19		
Assets at home			0.07		
	0:none	1.36 (1.03, 1.80)			
	1:one	0.88 (0.66, 1.16)			
	2:2-3	0.87 (0.67, 1.14)			
	3:4-5	Ref.			
Received TB treatment in the past 5 years		0.58 (0.31, 1.10)	0.10		
Hemoglobin, g/dL		0.95 (0.90, 1.00)	0.04	0.93 (0.89, 0.98)	0.007
Albumin, U/L		0.93 (0.86, 1.02)	0.13		
CD4 T-cells, 100 cells/ μ L		1.02 (0.99, 1.05)	0.12	1.04 (1.01, 1.07)	0.02
Depressed >2 weeks, ever		0.80 (0.61, 1.05)	0.11		
Hospitalization		1.30 (0.94, 1.80)	0.11		
Skin rash		1.32 (1.07, 1.64)	0.01		
Extrapulmonary TB		1.35 (0.93, 1.96)	0.11		

Height, cm		0.99 (0.98, 1.00)	0.08			
Weight, kg		0.99 (0.98, 1.00)	0.11			
WHO Body Mass Index (BMI) groups, kg/m ²			0.06			
	<16	0.81 (0.57, 1.16)				
	16-16.99	0.99 (0.76, 1.27)				
	17-18.49	Ref.				
	18.5-19.99	0.80 (0.63, 1.02)				
	20-21.99	0.75 (0.58, 0.96)				
	22+	1.05 (0.84, 1.30)				
Mid-Upper Arm Circumference (MUAC) quartiles, cm			0.02			
	<=21	Ref.				
	21.1-23.0	1.09 (0.90, 1.32)				
	23.1-24.9	0.81 (0.61, 1.08)				
	25+	0.82 (0.66, 1.03)				
p-values obtained using Binomial Regression; RR: Risk Ratio; 95% CI: 95% Confidence Interval						