



ELSEVIER

Contents lists available at ScienceDirect

## International Journal of Infectious Diseases

journal homepage: [www.elsevier.com/locate/ijid](http://www.elsevier.com/locate/ijid)

# Vitamin D supplementation to prevent tuberculosis infection in South African schoolchildren: multicenter phase 3 double-blind randomized placebo-controlled trial (ViDiKids)

Keren Middelkoop<sup>1,2</sup>, Justine Stewart<sup>1,2</sup>, Neil Walker<sup>3</sup>, Carmen Delport<sup>1</sup>, David A. Jolliffe<sup>4</sup>, Anna K. Coussens<sup>5,6</sup>, James Nuttall<sup>7</sup>, Jonathan C.Y. Tang<sup>8,9</sup>, William D. Fraser<sup>8,9</sup>, Christopher J. Griffiths<sup>3</sup>, Geeta Trilok Kumar<sup>10,11</sup>, Suzanne Filteau<sup>12</sup>, Richard L. Hooper<sup>3</sup>, Robert J. Wilkinson<sup>5,13,14</sup>, Linda-Gail Bekker<sup>1</sup>, Adrian R. Martineau<sup>4,\*</sup>

<sup>1</sup> Desmond Tutu HIV Centre, Institute of Infectious Diseases & Molecular Medicine, University of Cape Town, Observatory, South Africa

<sup>2</sup> Department of Medicine, University of Cape Town, Cape Town, South Africa

<sup>3</sup> Wolfson Institute of Population Health, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London, UK

<sup>4</sup> Blizard Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London, UK

<sup>5</sup> Wellcome Centre for Infectious Diseases Research in Africa, Institute of Infectious Disease and Molecular Medicine, University of Cape Town, Cape Town, South Africa

<sup>6</sup> Infectious Diseases and Immune Defense Division, Walter and Eliza Hall Institute of Medical Research, Parkville, Australia

<sup>7</sup> Paediatric Infectious Diseases Unit, Red Cross War Memorial Children's Hospital and the Department of Paediatrics and Child Health, University of Cape Town, Cape Town, South Africa

<sup>8</sup> Norwich Medical School, University of East Anglia, Norwich Research Park, Norwich, UK

<sup>9</sup> Departments of Laboratory Medicine, Clinical Biochemistry, and Departments of Diabetes and Endocrinology, Norfolk and Norwich University Hospital NHS Foundation Trust, Norwich, UK

<sup>10</sup> Delhi School of Public Health, Institute of Eminence, University of Delhi, Delhi, India

<sup>11</sup> Trivedi School of Biosciences, Ashoka University, Sonapat, India

<sup>12</sup> Faculty of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine, London, UK

<sup>13</sup> The Francis Crick Institute, London, UK

<sup>14</sup> Imperial College London, London, UK

## ARTICLE INFO

## Article history:

Received 3 February 2023

Revised 9 May 2023

Accepted 9 May 2023

## Keywords:

Vitamin D

Tuberculosis

Randomized controlled trial

Interferon-gamma release assay

## ABSTRACT

**Objectives:** To determine whether weekly oral supplementation with 10,000 IU vitamin D<sub>3</sub> for 3 years reduces the risk of sensitization to *M. tuberculosis* in South African schoolchildren aged 6–11 years with negative QuantiFERON-tuberculosis (TB) Gold Plus (QFT-Plus) assay results at baseline.

**Methods:** We conducted a phase 3 randomized placebo-controlled trial in 1682 children attending 23 primary schools in Cape Town. The primary outcome was a positive end-trial QFT-Plus result, analyzed using a mixed effects logistic regression model with the school of attendance included as a random effect. **Results:** 829 vs. 853 QFT-Plus-negative children were randomized to receive vitamin D<sub>3</sub> vs. placebo, respectively. Mean end-study 25(OH)D concentrations in participants randomized to vitamin D vs. placebo were 104.3 vs 64.7 nmol/l, respectively (95% confidence interval for difference, 37.6 to 41.9 nmol/l). A total of 76/667 (11.4%) participants allocated to vitamin D vs. 89/687 (13.0%) participants allocated to placebo tested QFT-Plus positive at 3-year follow-up (adjusted odds ratio 0.86, 95% confidence interval 0.62–1.19, *P* = 0.35).

**Conclusion:** Weekly oral supplementation with 10,000 IU vitamin D<sub>3</sub> for 3 years elevated serum 25(OH)D concentrations among QFT-Plus-negative Cape Town schoolchildren but did not reduce their risk of QFT-Plus conversion.

© 2023 The Authors. Published by Elsevier Ltd on behalf of International Society for Infectious Diseases. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>)

## Introduction

The global resurgence of tuberculosis after the COVID-19 pandemic has re-focused attention on strategies to achieve the World

\* Corresponding author: Tel: +44 207 882 7242.

E-mail address: [a.martineau@qmul.ac.uk](mailto:a.martineau@qmul.ac.uk) (A.R. Martineau).

Health Organization target of TB elimination by 2050 [1]. This target cannot be met without the implementation of measures to prevent the acquisition of *Mycobacterium tuberculosis* infection [2]. In countries with a high TB burden, such measures will need to focus particularly on schoolchildren, a group at particularly high risk of tuberculosis infection [3,4]. Existing efforts attempt to reduce transmission (e.g., by improved ventilation) [5] and boost immune resistance via repeat bacille Calmette-Guérin vaccination [6]. Vitamin D supplementation has also been proposed [7], based on the ability of 1,25-dihydroxyvitamin D (1,25[OH]<sub>2</sub>D), the biologically active vitamin D metabolite and steroid hormone) to induce innate antimycobacterial responses *in vitro* [8–11], reported associations between low circulating concentration of 25-hydroxyvitamin D (25[OH]D, the major circulating vitamin D metabolite and measure of vitamin D status) and increased susceptibility to *M. tuberculosis* infection [12–16], and findings of a randomized controlled trial (RCT) showing that oral vitamin D supplementation enhances the ability of whole blood to restrict mycobacterial growth *ex vivo* [17].

Recently, a phase III RCT conducted in Mongolian schoolchildren reported no effect of weekly oral administration of 350 µg (14,000 IU) vitamin D<sub>3</sub> on the risk of *M. tuberculosis* infection, as indicated by a positive QuantiFERON- tuberculosis (TB) Gold (QFT-Gold) assay, which detects CD4+ T cell interferon (IFN)-γ responses to *M. tuberculosis* antigens [18]. To determine whether this finding would be different in a lower-income setting with significantly higher TB transmission, we conducted a second phase III RCT among schoolchildren in Cape Town, South Africa, a city with one of the highest TB burdens in the world [19]. This trial differed in design from the sister trial in Mongolia by investigating the effects of a different dosing regimen (250 µg [10,000 IU] vitamin D<sub>3</sub> weekly) on QuantiFERON-TB Gold Plus (QFT-Plus) assay conversion. This fourth-generation assay represents an advance on QFT-Gold by allowing additional detection of CD8+ T cell IFN-γ responses to *M. tuberculosis* antigens, which may offer improved sensitivity for detection of immune conversion in recently exposed individuals [20] and children [21].

## Methods

### *Trial design, setting, approvals, and registration*

We conducted a multicenter phase III double-blind individually randomized placebo-controlled trial in 23 government schools in the Klipfontein district of Cape Town, South Africa. This peri-urban area is home to a socio-economically disadvantaged population who have a very high TB disease burden [4]. The trial was sponsored by Queen Mary University of London, approved by the University of Cape Town Faculty of Health Sciences Human Research Ethics Committee (Ref: 796/2015) and the London School of Hygiene and Tropical Medicine Observational/Interventions Research Ethics Committee (Ref: 7450-2) and registered on the South African National Clinical Trials Register (DOH-27-0916-5527) and ClinicalTrials.gov (ref NCT02880982). Trial monitoring was performed by OnQ Research (Pty) Ltd (Randburg, South Africa).

### *Participants*

Inclusion criteria were enrollment in Grades I–III at a participating school; age 6 to 11 years at screening; and written informed assent/consent to participate provided by children and their parent/legal guardian, respectively. Exclusion criteria were a history of previous latent TB infection, active TB disease, or any chronic illness other than asthma (including known or suspected HIV infection) before enrollment; use of any regular medication other than asthma medication; use of vitamin D supplements at a dose of

more than 400 IU/day in the month before enrollment; plans to move away from study area within 3 years of enrollment; inability to swallow a placebo soft gel capsule with ease; and clinical evidence of rickets or a positive QFT-Plus assay result at screening.

### *Enrollment*

Parents or legal guardians were invited to provide written informed consent for their child to participate in the trial during a home visit. If they agreed, they were asked to provide details regarding their child's eligibility to participate and risk factors for tuberculosis infection. Potentially eligible children were then invited to provide written assent to participate in a school-based visit. If they agreed, a clinically trained member of the study team screened them for symptoms and signs of rickets, a blood sample was taken for a QFT-Plus assay, and separation and storage of serum for determination of 25(OH)D concentrations as described below. Participants were reviewed when baseline QFT-Plus results were available. Those with a positive result were excluded from the trial and screened for active TB; preventive therapy was not routinely offered where this was excluded, in line with local guidelines. Those with an indeterminate result were excluded from the trial without screening for active TB. Those with a negative result were deemed eligible to participate and underwent measurement of weight (using a digital floor scale, Charder Medical) and height (using a portable HM200P stadiometer, Charder Medical). The first 200 eligible participants to be enrolled were also invited to participate in a safety sub-study, and those who agreed also provided a baseline urine sample for the determination of urinary calcium and creatinine concentration at this visit.

### *Randomization and blinding*

Eligible participants were individually randomized to receive a weekly capsule containing vitamin D<sub>3</sub> or a placebo for 3 years, with a one-to-one allocation ratio. Randomization was stratified by school of attendance (a potential predictor of risk of QFT conversion) as described in Supplementary Material. Treatment allocation was concealed from participants, care providers, and all trial staff (including senior investigators and those assessing outcomes) so that the double-blind was maintained.

### *Intervention*

Study medication comprised a 3-year course of weekly soft gel capsules manufactured by the Tishcon Corporation (Westbury, NY, USA), containing either 0.25 mg (10,000 international units) cholecalciferol (vitamin D<sub>3</sub>) in olive oil (intervention arm) or olive oil without any vitamin D<sub>3</sub> content (placebo arm). Active and placebo capsules had identical appearance and taste. Capsules were taken under direct observation of study staff during school term. During the summer holidays (8 weeks), packs containing eight doses of study medication were provided for administration by parents, together with a participant diary. After shorter school holidays (≤4 weeks), and/or if participants missed one or more doses of study medication during term time, up to 4 'catch-up' doses were administered at the first weekly visit attended after the missed dose(s). During the initial national lockdown for COVID-19 in South Africa (March 27 to May 01, 2020), participants did not receive any study medication. During subsequent school closures due to COVID-19, two rounds of 8-week holiday packs were provided to participants, which were sufficient to cover their requirements until schools reopened.

At weekly study visits during school terms, the study team captured data on adverse events and supervised the administration of study capsules. Weight and height were measured at 3-monthly

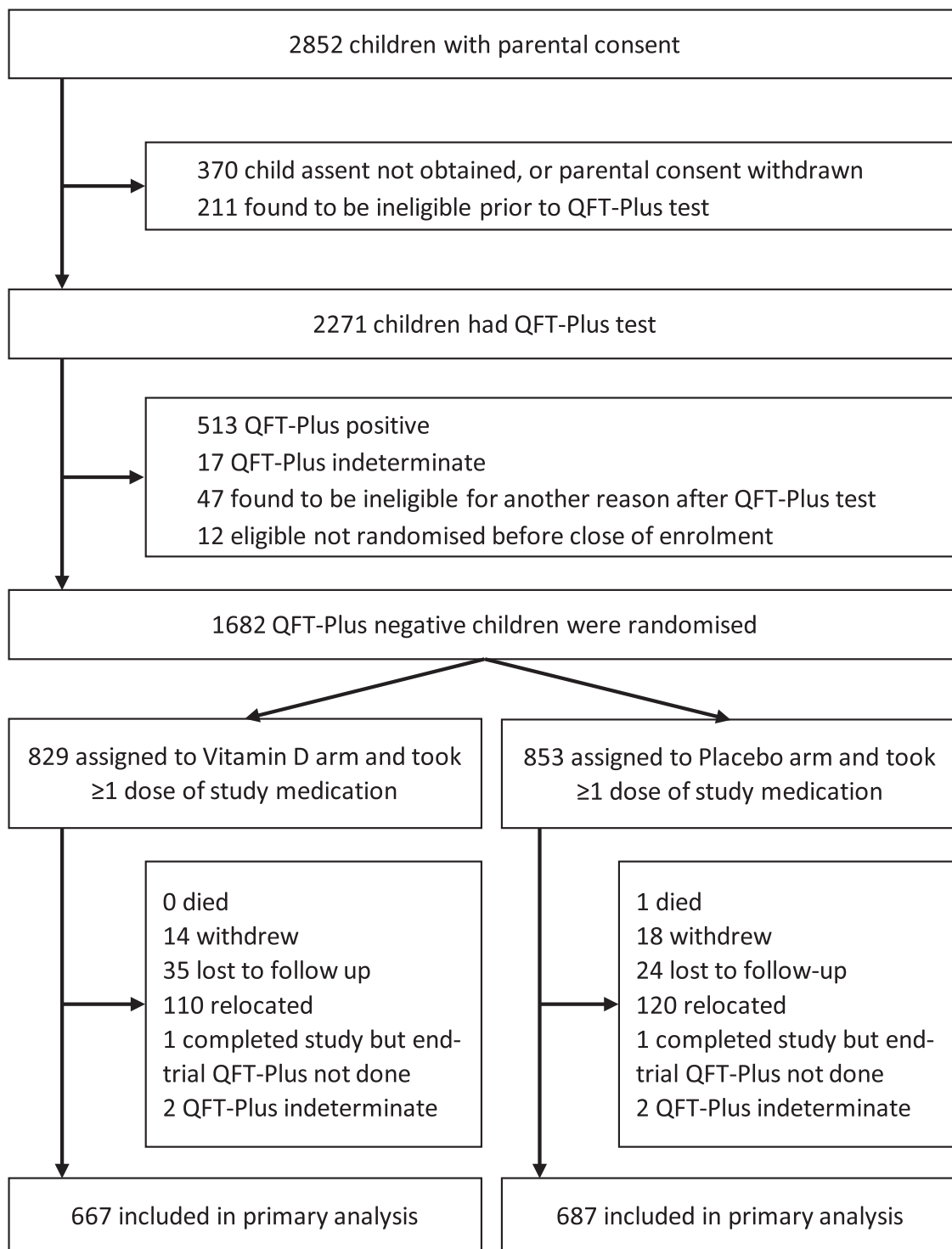


Figure 1. Participant flow. QFT-Plus, QuantiFERON tuberculosis Gold Plus.

intervals. At annual intervals, history of TB exposure was elicited and physical examination for cervical lymphadenopathy was performed. Any participant with symptoms suggestive of hypercalcemia or TB was evaluated by a clinical member of the team and referred for further investigation or management as appropriate. At the 36-month visit, all participants were invited to provide a blood sample for QFT-Plus testing and separation and storage of serum for biochemical analyses. In addition to these assessments, safety sub-study participants were invited to give additional blood

and urine samples at 6-, 12-, 24- and 36-month visits for determination of serum concentrations of 25(OH)D<sub>3</sub>, albumin and calcium and urine concentrations of calcium and creatinine.

Outcomes

The primary outcome was the QuantiFERON-TB Gold Plus result at the manufacturer-recommended 0.35 IU/ml threshold at the end of the study. Secondary efficacy outcomes were the QuantiFERON-

TB Gold Plus result at the 4.0 IU/ml IFN- $\gamma$  threshold at the end of the study (previously reported as denoting sustained conversion) [6]; antigen-stimulated IFN- $\gamma$  concentration; incident TB disease; and serum 25(OH)D<sub>3</sub> concentrations at the end of the study. Safety outcomes were death, serious adverse events, adverse events leading to discontinuation of study medication, and other monitored safety conditions: hypercalcemia (serum-adjusted calcium concentration >2.75 mmol/l, confirmed on two samples), hypervitaminosis D (25(OH)D<sub>3</sub> concentration >220 nmol/l) and renal stones. Additional outcomes assessed in safety sub-study participants were serum-adjusted calcium concentrations of 25(OH)D<sub>3</sub> at 6, 12, and 24 months, and urinary calcium: creatinine ratios at 6, 12, 24, and 36 months.

#### Laboratory assessments

QFT-Plus assays were performed by the Bio Analytical Research Corporation South Africa (Johannesburg, South Africa) according to the manufacturer's instructions, with positivity adjudicated at 0.35 IU/ml and 4.0 IU/ml IFN- $\gamma$  thresholds. Serum concentrations of 25(OH)D<sub>3</sub> were measured at the University of East Anglia (Norwich, UK) using liquid chromatography-tandem mass spectrometry (LC-MS/MS) as previously described [22]. 25(OH)D<sub>3</sub> was calibrated using standard reference material SRM972a from the National Institute of Science and Technology (NIST), and the assay showed linearity between 0 and 200 nmol/l. The inter/intra-assay coefficient of variation (CV) across the assay range was  $\leq$ 9% and the lower limit of quantification was 0.1 nmol/l. The assay showed <6% accuracy bias against NIST reference method on the vitamin D external quality assessment (DEQAS) scheme (<http://www.deqas.org/>; accessed on November 30, 2022). Serum concentrations of total calcium, albumin, and creatinine and urine concentrations of total calcium and creatinine were measured at the University of East Anglia by spectrophotometric methods on the Cobas c501 platform (Roche Diagnostics, Germany) according to the manufacturer's instructions. The inter-assay CVs for total calcium and albumin were  $\leq$  2.1% across the assay working ranges of 0.2 to 7.5 mmol/l and 2 to 60 g/l. Albumin-adjusted calcium was calculated as  $(4 - \text{albumin concentration in g/dl}) \times 0.8 + \text{total serum calcium concentration in mg/dl}$ . Urine creatinine was analyzed using a Roche 2nd generation assay based on the Jaffé method. The inter-assay CV ranged from 1.3% to 2.1% and the intra-assay CV ranged between 1.6% to 4.0% across the assay working range of 0.015 to 55 mmol/l. Urinary calcium: creatinine ratios were calculated using molar concentrations of each analyte.

#### Sample size

Assuming a 3.5% annual risk of TB infection, 20% loss to follow-up, and a 5% risk of an indeterminate QFT-Plus assay result at the end of the study, we calculated that a total of 5400 participants would need to be randomized to detect a 25% reduction in the proportion of children with a positive QFT-Plus assay result at 3-year follow-up with 80% power and 5% type 1 error. Target sample size was not achieved because of poorer-than-expected recruitment and a lack of funding to extend the recruitment period. Enrollment was terminated in March 2019, with the agreement of the independent Trial Steering Committee, at which point a total of 1682 participants had been randomized.

#### Statistical analyses

Statistical analyses were performed using Stata software (Version 17.0; StataCorp, College Station, Texas, United States) according to intention to treat. Effects of treatment on dichotomous outcomes were estimated by fitting allocation (vitamin D vs placebo)

as the sole fixed effect in a mixed-effects logistic regression model with school of attendance included as a random effect (intercept). Results are reported as odds ratios with 95% confidence intervals (CIs). A secondary analysis of the primary outcome was conducted including the following pre-specified list of covariates anticipated to associate with the risk of QFT-Plus conversion: age, sex, month of sampling, household TB contact, baseline deseasonalized 25(OH)D concentration and household exposure to tobacco smoke.

Effects of allocation to vitamin D vs placebo on continuous outcomes were estimated using mixed effects linear regression with school of attendance as a random effect and results reported as treatment differences with 95% CIs. Concentrations of IFN- $\gamma$  in supernatants of end-study QFT-Plus assays in test-positive participants with were log-transformed before analysis. Baseline z-scores for body mass index-for-age and height-for-age were calculated using the WHO 2007 Z-Score Lambda, Mu, and Sigma methods [23]. Season-adjusted (deseasonalized) values for baseline 25(OH)D were calculated for each participant using a sinusoidal model as previously described [15]. Pre-specified sub-group analyses were conducted to determine whether the effect of vitamin D supplementation on end-trial QFT-Plus status was modified by baseline vitamin D status. These were performed by repeating primary efficacy analyses with the inclusion of an interaction term between allocation (to vitamin D vs placebo) and baseline serum 25(OH)D<sub>3</sub> concentration (<75 vs  $\geq$ 75 nmol/l), with presentation of the P-value associated with this interaction term. Pre-specified sensitivity analyses excluding data from participants who took fewer than 80% of doses of study medication were not conducted, as adherence to study medication during school closures due to COVID-19 lockdowns was not assessed. Interim safety assessments, where Independent Data Monitoring Committee (IDMC) members reviewed accumulating serious adverse event data (all participants) and biochemical outcomes (safety sub-study participants only) by study arm, were performed at 6-monthly intervals. At each review, the DSMB recommended continuation of the trial. No interim efficacy analysis was performed.

## Results

### Participants

Parental consent was obtained for 2852 children from March 2017 to March 2019. A total of 2271 children underwent QFT-Plus testing, of whom 1682 (74.1%) had negative results and were randomly assigned to receive vitamin D<sub>3</sub> (829 participants) or placebo (853 participants; Figure 1). Reasons for ineligibility are provided in Table S1. The mean age of the children at enrollment was 8.9 years (SD 1.4), 52.4% were female and 97.9% were of Xhosa ethnic origin. The mean deseasonalized serum 25(OH)D<sub>3</sub> level at baseline was 71.2 nmol/l (SD 14.8), and 63.2% of participants had deseasonalized serum 25(OH)D<sub>3</sub> concentration <75 nmol/l. Participant characteristics were balanced between those randomized to vitamin D vs placebo (Table 1).

The median duration of follow-up was 3.16 years (interquartile range, 2.83 to 3.38 years) and was not different between the two study arms. Valid end-trial QFT-Plus results were obtained for 1354 participants (80.5% of those randomized) who were included in the analysis of the primary outcome. Mean end-trial serum 25(OH)D<sub>3</sub> concentrations were higher among children randomized to receive vitamin D versus placebo (104.3 vs 64.7 nmol/l, respectively; mean difference 39.7 nmol/l, 95% CI for difference 37.6 to 41.9 nmol/l; Figure 2). At the end of the trial, 90.4% of children in the vitamin D group and 25.0% in the placebo group had serum 25(OH)D<sub>3</sub> concentration  $\geq$ 75 nmol/l ( $P < 0.001$ ).

**Table 1**  
Participants' baseline characteristics by allocation.

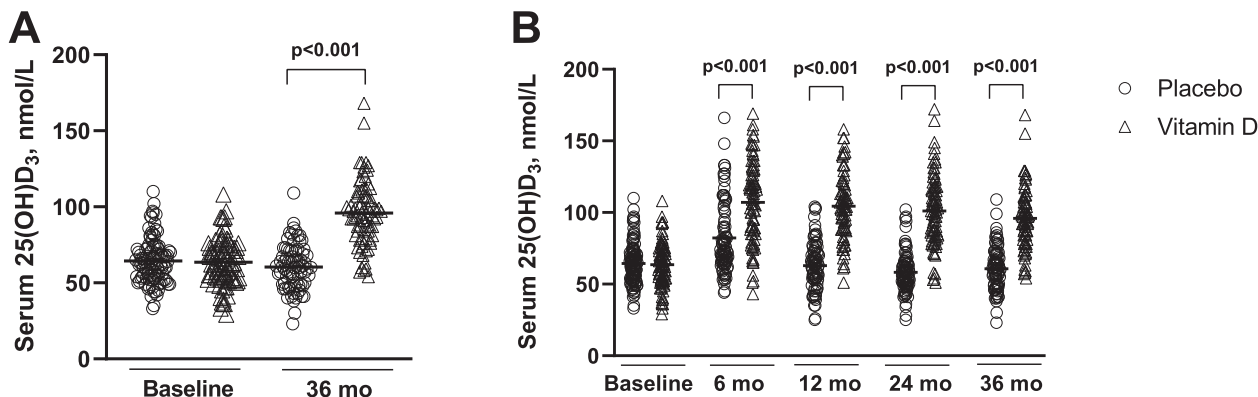
		Overall (n = 1682)	Vitamin D arm (n = 829)	Placebo arm (n = 853)
Mean age, years (SD)		8.9 (1.4)	8.9 (1.4)	8.8 (1.3)
Female sex, n (%) <sup>a</sup>		880 (52.4)	437 (52.8)	443 (51.9)
Ethnic origin <sup>a</sup>	Xhosa, n (%)	1615 (97.9)	788 (97.3)	827 (98.5)
	Other, n (%)	35 (2.1)	22 (2.7)	13 (1.5)
Type of residence	Brick, n (%)	867 (51.5)	423 (51.0)	444 (52.1)
	Informal, n(%)	815 (48.5)	406 (49.0)	409 (47.9)
Parental education <sup>a,b</sup>	Primary school, n (%)	60 (3.6)	34 (4.1)	26 (3.1)
	Secondary school or higher, n (%)	1618 (96.4)	792 (95.9)	826 (96.9)
Mean monthly household income, 1000 South African Rand (SD)		1.9 (2.2)	1.8 (2.1)	2.0 (2.2)
Household environmental tobacco smoke, n (%)		228 (13.6)	127 (15.3)	101 (11.8)
Previous household pulmonary tuberculosis contact, n (%)		245 (14.6)	130 (15.7)	115 (13.5)
Bacille Calmette-Guerin scar, n (%)		1634 (97.1)	807 (97.3)	827 (97.0)
Mean body mass index-for-age z-score (SD) <sup>a</sup>		0.3 (1.1)	0.3 (1.1)	0.3 (1.0)
Mean height-for-age z-score (SD) <sup>a</sup>		-0.6 (1.2)	-0.6 (1.3)	-0.5 (1.1)
Mean serum 25(OH)D <sub>3</sub> concentration, nmol/l (s.d.) <sup>a,c</sup>		71.2 (14.8)	71.2 (14.5)	71.1 (15.0)
Serum 25(OH)D <sub>3</sub> concentration, category <sup>a,c</sup>	Missing (N (%))	318 (18.9)	159 (19.2)	159 (18.6)
	<25 nmol/l, n (%)	1 (0.1)	0 (0.0)	1 (0.1)
	≥ 25 nmol/l & < 50 nmol/l, n (%)	74 (5.4)	34 (5.1)	40 (5.8)
	≥50 nmol/l & < 75 nmol/l, n (%)	787 (57.7)	394 (58.8)	393 (56.6)
	≥75 nmol/l, n (%)	502 (36.8)	242 (36.1)	260 (37.5)

**Abbreviations:** 25(OH)D<sub>3</sub>, 25-hydroxyvitamin D<sub>3</sub>.

<sup>a</sup> Missing data (sex, n = 2 vitamin D arm, n = 0 placebo arm; ethnicity, n = 19 vitamin D arm, n = 13 placebo arm; parental education, n = 3 vitamin D arm, n = 1 placebo arm; body mass index-for-age z-score, n = 2 vitamin D arm, n = 0 placebo arm; height-for-age z-score, n = 2 vitamin D arm, n = 0 placebo arm; serum 25(OH)D<sub>3</sub> concentration, n = 159 vitamin D arm, n = 159 placebo arm).

<sup>b</sup> highest level of education of at least one parent;

<sup>c</sup> Deseasonalized values.



**Figure 2.** Serum 25(OH)D<sub>3</sub> concentrations by allocation and time point. (a) serum 25(OH)D<sub>3</sub> concentrations by allocation at baseline and 3-year follow-up, all participants. (b) serum 25(OH)D<sub>3</sub> concentrations by allocation at baseline and 6-month, 1-year, 2-year and 3-year follow-up, safety sub-study participants. Horizontal bars denote mean values. 25(OH)D<sub>3</sub>, 25-hydroxyvitamin D<sub>3</sub>.

*QuantiferON-tuberculosis Gold Plus (QFT-Plus) outcomes*

The proportion of participants with a positive end-trial QFT-Plus result at the 0.35 IU/ml IFN-γ threshold (primary outcome) was similar for those randomized to vitamin D vs placebo (76/667 [11.4%] vs 89/687 [13.0%] respectively; adjusted odds ratio [aOR] 0.86, 95% CI 0.62 to 1.19, P = 0.35; Table 2). The corresponding result from the secondary multiple regression analysis described in Methods was as follows: aOR 0.82, 95% CI 0.59 to 1.15, P = 0.25. This analysis involved multiple imputations (10 imputed analyses) on baseline deseasonalized 25(OH)D concentration (regressed on age, sex, and household TB exposure) since 260/1354 [19.2%] 260/1354 (19.2%) of observations were missing for this variable. Proportions of children with a positive end-trial QFT-Plus result at the 4.0 IU/ml IFN-γ threshold were also similar for those randomized to vitamin D vs placebo (25/667 [3.7%] vs 19/687 [2.8%] respectively; aOR 1.34, 95% CI 0.73 to 2.46, P = 0.35; Table 2). Subgroup analyses evaluating the effects of the intervention among participants with lower vs higher baseline 25(OH)D<sub>3</sub> concentra-

tions (<75 vs ≥75 nmol/l) revealed no evidence to suggest effect modification by baseline vitamin D status, either for QFT-Plus conversion at the 0.35 IU/ml IFN-γ threshold (P for interaction 0.38) or the 4.0 IU/ml IFN-γ threshold (P for interaction 0.74; Table 2). In the subset of participants with positive end-trial QFT-Plus results at the 0.35 IU/ml IFN-γ threshold, allocation to vitamin D vs placebo did not influence mean antigen-stimulated IFN-γ concentrations in supernatants, either overall or in sub-groups defined according to baseline 25(OH)D<sub>3</sub> concentration (Table S2, Supplementary Material).

*Active tuberculosis*

Incident active TB was diagnosed during follow-up in 0/829 participants randomized to vitamin D vs 3/853 (0.4%) participants randomized to placebo. The associated odds ratio was not calculated due to the absence of active TB cases arising among participants allocated to vitamin D.



**Table 2**Proportions of participants with a positive end-study QFT-Plus result by allocation: overall and by baseline serum 25(OH)D<sub>3</sub> concentration.

		Vitamin D arm, n/N (%)	Placebo arm, n/N (%)	Adjusted odds ratio (95% confidence interval) <sup>a</sup>	P-value <sup>b</sup>	P for interaction <sup>c</sup>
Proportion QFT-Plus positive at the 0.35 IU/ml IFN- $\gamma$ threshold (primary outcome)	Overall	76/667 (11.4)	89/687 (13.0)	0.86 (0.62 to 1.19)	0.35	–
	Baseline 25(OH)D <sub>3</sub> <75 nmol/l <sup>d</sup>	36/338 (10.7)	45/356 (12.9)	0.80 (0.50 to 1.28)	0.35	0.38
	Baseline 25(OH)D <sub>3</sub> ≥75 nmol/l <sup>d</sup>	27/195 (13.8)	26/205 (12.7)	1.11 (0.62 to 1.98)	0.73	
Proportion QFT-Plus positive at the 4.0 IU/ml IFN- $\gamma$ threshold (secondary outcome)	Overall	25/667 (3.7)	19/687 (2.8)	1.34 (0.73 to 2.46)	0.35	–
	Baseline 25(OH)D <sub>3</sub> <75 nmol/l <sup>d</sup>	12/338 (3.6)	11/356 (3.1)	1.14 (0.49 to 2.64)	0.76	0.74
	Baseline 25(OH)D <sub>3</sub> ≥75 nmol/l <sup>d</sup>	7/195 (3.6)	5/205 (2.4)	1.49 (0.46 to 4.78)	0.50	

Abbreviations: 25(OH)D<sub>3</sub>, 25-hydroxyvitamin D<sub>3</sub>; IFN- $\gamma$ , Interferon-gamma; QFT-Plus, QuantiFERON tuberculosis gold plus.<sup>a</sup> Adjusted for school of attendance;<sup>b</sup> P for within-group or within-subgroup comparison between study arms;<sup>c</sup> Applicable to sub-group analyses only;<sup>d</sup> Deaseasonalized values.**Table 3**

Adverse events by allocation.

	Vitamin D arm		Placebo arm		
	No. of events	Participants with ≥1 event	No. of events	Participants with ≥1 event	
Death <sup>a</sup>	0	0	1	1	
Non-fatal serious adverse events <sup>a</sup>	11	8	9	8	
Non-fatal adverse event leading to discontinuation of study medication	0	0	0	0	
Other monitored safety conditions	Hypercalcemia <sup>b</sup>	0	0	0	
	Hypervitaminosis D <sup>c</sup>	0	0	0	
	Renal stones	0	0	0	0

<sup>a</sup> Details in Appendix Table S4;<sup>b</sup> Defined as serum adjusted calcium concentration >2.75 mmol/l confirmed on repeat testing;<sup>c</sup> Defined as serum 25-hydroxyvitamin-D<sub>3</sub> concentration >220 nmol/l.

### Biochemical outcomes, safety sub-study

Among children participating in the safety sub-study, mean serum 25(OH)D<sub>3</sub> concentrations were consistently higher among those allocated to vitamin D vs placebo at 6, 12, 24, and 36 months post-randomization (Figure 2, Table S3). This was not associated with any inter-arm differences in mean adjusted calcium concentration or mean urinary calcium: creatinine molar ratio at any follow-up time point (Table S3). No safety sub-study participant experienced hypervitaminosis D (serum 25[OH]D<sub>3</sub> concentration >220 nmol/l), hypercalcemia (adjusted serum calcium concentration >2.75 mmol/l), or hypercalciuria (urinary calcium: creatinine molar ratio >1.00) at any follow-up timepoint.

### Adverse events

One child (allocated to placebo) died during the trial, and 16 children (eight in the vitamin D group and eight in the placebo group) had one or more non-fatal serious adverse events (Table 3, Supplementary Table S4). None of these events was adjudged to be related to administration of vitamin D or placebo.

### Discussion

We report findings of the first RCT of vitamin D supplementation to prevent tuberculosis infection to be conducted in Africa. In a cohort of schoolchildren without evidence of tuberculosis infection at baseline, administration of oral vitamin D<sub>3</sub> at a weekly dose of 250  $\mu$ g (10,000 IU) for a period of 3 years was found to be safe and effective in elevating circulating 25(OH)D concentrations.

However, this intervention did not reduce the risk of QFT-Plus conversion, either at the standard threshold of 0.35 IU/ml IFN- $\gamma$  or at a higher threshold (4.0 IU/ml IFN- $\gamma$ ) that has been reported to associate with sustained conversion [6]. Effects of the intervention were not modified by baseline vitamin D status.

Null findings from this trial contrast with positive results of observational studies reporting independent associations between low vitamin D status and increased risk of tuberculosis infection in South Africa [12] and elsewhere [13–16]. However, they are consistent with the results of another phase III trial of vitamin D supplementation for tuberculosis prevention in schoolchildren, conducted in Mongolia, in which a 3-year course of weekly oral vitamin D supplementation at a dose of 350  $\mu$ g (14,000 IU) did not influence risk of QFT conversion or active tuberculosis in schoolchildren with very low baseline vitamin D status [18]. Of note, another large RCT of oral vitamin D supplementation in HIV-infected adults in Tanzania reported no effect of the intervention on the risk of incident pulmonary tuberculosis [24]. Null results from this RCT and our own should be interpreted in the context of participants' relatively high baseline vitamin D status: their findings should not be generalized to populations with significantly lower baseline vitamin D status.

Inconsistent findings from observational studies vs RCT in this field may reflect the fact that observational studies are more susceptible to confounding or (in the case of cross-sectional studies) reverse causality, whereby *M. tuberculosis* may reduce circulating 25(OH)D concentrations by perturbing host vitamin D metabolism [25] or by metabolizing cholecalciferol itself [26]. However, RCT also has methodological limitations. Null results from our study may reflect type II error, particularly for the secondary outcome of active TB, which arose in just three participants. The question of

whether vitamin D may reduce risk of active TB in children therefore remains open [27–29]. For the primary outcome, this problem was offset by a higher-than-expected incidence of QFT-Plus conversion (12.2% observed vs 10.1% anticipated in the power calculation), and lower-than-expected incidence of indeterminate QFT-Plus results at follow-up (0.2% observed vs 5% anticipated in the power calculation). In addition, administration of study medication was suspended for 8 weeks because of school closures triggered by the COVID-19 pandemic. This interruption occurred late in the trial when participants randomized to intervention would have achieved steady-state vitamin D status, and the long half-life of 25(OH)D in the circulation (1–2 months) [30] makes it likely that an inter-arm difference in vitamin D status was maintained during this break. The relatively short period of interruption (accounting for just 4.9% of median follow-up time), and demonstration of clear differences in end-study vitamin D status between children randomized to intervention vs control provide reassurance that the interruption did not have a sustained impact on 25(OH)D levels in the intervention arm. Profound vitamin D deficiency (25[OH]D <25 nmol/l) at baseline was rare, so our findings should not be generalized to populations with very low vitamin D status: however, we highlight that null findings from the sister trial in Mongolia [18], where profound vitamin D deficiency was prevalent at baseline, do not suggest a protective effect of vitamin D against tuberculosis infection even in this sub-group.

Our study also has several strengths. Chief among them was the RCT design, which was effective in distributing risk factors for tuberculosis infection evenly between arms (Table 1). Direct observation of administration of study medication during term time, with parental administration during holiday periods, was effective in achieving a high degree of adherence, reflected by significant increases in serum 25(OH)D concentrations observed among participants randomized to intervention. A further strength was our use of the QFT-Plus assay, which (in contrast to the QFT-Gold assay employed in the trial by Ganmaa *et al.*) [18] includes an additional blood tube containing peptides targeted to the induction of IFN- $\gamma$  responses from CD8+ cytotoxic T lymphocytes [31]. This may offer improved sensitivity for the detection of tuberculosis infection in recently exposed individuals [20] and in young children [21].

In conclusion, this phase III RCT showed that a 3-year course of weekly oral vitamin D supplementation was safe and effective in elevating serum 25(OH)D concentrations among South African schoolchildren aged 6–11 years at baseline. However, the intervention did not reduce risk of acquiring *M. tuberculosis* infection, as evidenced by QFT-Plus assay conversion.

## Declarations of Competing Interest

ARM declares receipt of funding in the last 36 months to support vitamin D research from the following companies who manufacture or sell vitamin D supplements: Pharma Nord Ltd, DSM Nutritional Products Ltd, Thornton & Ross Ltd and Hyphens Pharma Ltd. ARM also declares receipt of vitamin D capsules for clinical trial use from Pharma Nord Ltd, Synergy Biologics Ltd and Cytoplant Ltd; support for attending meetings from Pharma Nord Ltd and Abiogen Pharma Ltd; receipt of consultancy fees from DSM Nutritional Products Ltd and Qiagen Ltd; receipt of a speaker fee from the Linus Pauling Institute; participation on Data and Safety Monitoring Boards for the VITALITY trial (Vitamin D for Adolescents with HIV to reduce musculoskeletal morbidity and immunopathology, Pan African Clinical Trials Registry ref PACTR20200989766029) and the Trial of Vitamin D and Zinc Supplementation for Improving Treatment Outcomes Among COVID-19 Patients in India (ClinicalTrials.gov ref NCT04641195); and unpaid work as a Programme Committee member for the Vitamin D Workshop. All other authors declare that they have no competing interests.

## Funding

This research was funded by the United Kingdom (UK) Medical Research Council (MR/M026639/1 awarded to ARM). RJW was supported by Wellcome (104803, 203135). He also received support from the Francis Crick Institute which is funded by Cancer Research UK (FC2112), the UK Medical Research Council (FC2112), and Wellcome (FC2112).

## Acknowledgements

We thank all the children who participated in the trial, and their parents/guardians; members of the Independent Data Monitoring Committee (Prof Guy Thwaites, Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam [Chair]; Prof John Pettifor, University of the Witwatersrand, Johannesburg, South Africa; and Prof Sarah Walker, MRC Clinical Trials Unit, London, UK); and members of the Trial Steering Committee (Prof Beate Kampmann, London School of Hygiene and Tropical Medicine, London, UK [Chair]; Prof Ashraf Coovadia, University of the Witwatersrand, Johannesburg, South Africa; Dr Karen Jennings, City Health, Cape Town, South Africa; and Dr Guy de Bruyn, Sanofi Pasteur, Swiftwater PA USA). For the purposes of open access, the author has applied a CC-BY public copyright to any author-accepted manuscript arising from this submission.

## Author contributors

ARM conceived the study. KM, AKC, JN, GTK, SF, RJW, RHL, LGB, and ARM contributed to study design and protocol development. KM led on trial implementation, with support from JS, CD, DAJ, JN, LGB, and ARM. JCYT and WDF performed and supervised the conduct of biochemical assays. NW, ARM, and RLH drafted the statistical analysis plan. DAJ, KM, JS, NW, and CD managed data. NW, KM, and JS accessed, verified, and analyzed the data underlying the study. KM and ARM wrote the first draft of the trial report. All authors made substantive comments thereon and approved the final version for submission.

## Data sharing

Anonymized data may be requested from the corresponding author to be shared subject to terms of research ethics committee approval.

## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2023.05.010.

## References

- [1] World Health Organization *Global tuberculosis report 2022*. Geneva: World Health Organization; 2022.
- [2] Dye C, Williams BG. Eliminating human tuberculosis in the twenty-first century. *J R Soc Interface* 2008;**5**:653–62. doi:10.1098/rsif.2007.1138.
- [3] Wood R, Liang H, Wu H, Middelkoop K, Oni T, Rangaka MX, Wilkinson RJ, et al. Changing prevalence of tuberculosis infection with increasing age in high-burden townships in South Africa. *Int J Tuberc Lung Dis* 2010;**14**:406–12.
- [4] Middelkoop K, Bekker LG, Liang H, Aquino LD, Sebastian E, Myer L, et al. Force of tuberculosis infection among adolescents in a high HIV and TB prevalence community: a cross-sectional observation study. *BMC Infect Dis* 2011;**11**:156. doi:10.1186/1471-2334-11-156.
- [5] Richardson ET, Morrow CD, Kalil DB, Bekker LG, Wood R. Shared air: a renewed focus on ventilation for the prevention of tuberculosis transmission. *PLoS One* 2014;**9**:e96334. doi:10.1371/journal.pone.0096334.
- [6] Nemes E, Geldenhuys H, Rozot V, Rutkowski KT, Ratangee F, Bilek N, et al. Prevention of *M. tuberculosis* infection with H4:IC31 vaccine or BCG revaccination. *N Engl J Med* 2018;**379**:138–49. doi:10.1056/NEJMoa1714021.

- [7] Davies PD, Martineau AR. Vitamin D and tuberculosis: more effective in prevention than treatment? *Int J Tuberc Lung Dis* 2015;**19**:876–7. doi:10.5588/ijtld.15.0506.
- [8] Rook GA, Steele J, Fraher L, Barker S, Karmali R, O'riordan J, et al. Vitamin D3, gamma interferon, and control of proliferation of Mycobacterium tuberculosis by human monocytes. *Immunology* 1986;**57**:159–63.
- [9] Liu PT, Stenger S, Li H, Wenzel L, Tan BH, Krutzik SR, et al. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. *Science* 2006;**311**:1770–3. doi:10.1126/science.1123933.
- [10] Martineau AR, Wilkinson KA, Newton SM, Floto RA, Norman AW, Skolimowska K, et al. IFN-gamma and TNF-independent vitamin D-inducible human suppression of mycobacteria: the role of cathelicidin LL-37. *J Immunol* 2007;**178**:7190–8. doi:10.4049/jimmunol.178.11.7190.
- [11] Yuk JM, Shin DM, Lee HM, Yang CS, Jin HS, Kim KK, et al. Vitamin D3 induces autophagy in human monocytes/macrophages via cathelicidin. *Cell Host Microbe* 2009;**6**:231–43. doi:10.1016/j.chom.2009.08.004.
- [12] Martinez L, Ncayiyana JR, Goddard E, Botha M, Workman L, Burd T, et al. Vitamin D concentrations in infancy and the risk of tuberculosis disease in childhood: a prospective birth cohort in Cape Town, South Africa. *Clin Infect Dis* 2022;**74**:2036–43. doi:10.1093/cid/ciab735.
- [13] Gibney KB, MacGregor L, Leder K, Torresi J, Marshall C, Ebeling PR, et al. Vitamin D deficiency is associated with tuberculosis and latent tuberculosis infection in immigrants from sub-Saharan Africa. *Clin Infect Dis* 2008;**46**:443–6. doi:10.1086/525268.
- [14] Arnedo-Pena A, Juan-Cerdan JV, Romeu-Garcia MA, Garcia-Ferrer D, Holguin-Gomez R, Iborra-Millet J, et al. Vitamin D status and incidence of tuberculosis infection conversion in contacts of pulmonary tuberculosis patients: a prospective cohort study. *Epidemiol Infect* 2015;**143**:1731–41. doi:10.1017/S0950268814002386.
- [15] Ganmaa D, Khudyakov P, Buyanjargal U, Jargalsaikhan B, Baigal D, Munkhjarjal O, et al. Prevalence and determinants of QuantiFERON-diagnosed tuberculosis infection in 9810 Mongolian schoolchildren. *Clin Infect Dis* 2019;**69**:813–19. doi:10.1093/cid/ciy975.
- [16] Wang CY, Hu YL, Wang YH, Chen CH, Lai CC, Huang KL. Association between vitamin D and latent tuberculosis infection in the United States: NHANES, 2011–2012. *Infect Drug Resist* 2019;**12**:2251–7. doi:10.2147/IDR.S213845.
- [17] Martineau AR, Wilkinson RJ, Wilkinson KA, Newton SM, Kampmann B, Hall BM, et al. A single dose of vitamin D enhances immunity to mycobacteria. *Am J Respir Crit Care Med* 2007;**176**:208–13. doi:10.1164/rccm.200701-0070C.
- [18] Ganmaa D, Uyanga B, Zhou X, Gantsetseg G, Delgerekh B, Enkhmaa D, et al. Vitamin D supplements for prevention of tuberculosis infection and disease. *N Engl J Med* 2020;**383**:359–68. doi:10.1056/NEJMoa1915176.
- [19] Hermans S, Horsburgh CR Jr, Wood R. A century of tuberculosis epidemiology in the northern and southern hemisphere: the differential impact of control interventions. *PLoS One* 2015;**10**:e0135179. doi:10.1371/journal.pone.0135179.
- [20] Nikolova M, Markova R, Drenska R, Muhtarova M, Todorova Y, Dimitrov V, et al. Antigen-specific CD4+ and CD8+ positive signatures in different phases of Mycobacterium tuberculosis infection. *Diagn Microbiol Infect Dis* 2013;**75**:277–81. doi:10.1016/j.diagmicrobio.2012.11.023.
- [21] Lancioni C, Nyendak M, Kiguli S, Zalwango S, Mori T, Mayanja-Kizza H, et al. CD8+ T cells provide an immunologic signature of tuberculosis in young children. *Am J Respir Crit Care Med* 2012;**185**:206–12. doi:10.1164/rccm.201107-1355OC.
- [22] Middelkoop K, Walker N, Stewart J, Delpont C, Jolliffe DA, Nuttall J, et al. Prevalence and determinants of vitamin D deficiency in 1825 Cape Town primary schoolchildren: a cross-sectional study. *Nutrients* 2022;**14**:1263. doi:10.3390/nu14061263.
- [23] Ganmaa D, Bromage S, Khudyakov P, Erdenenbaatar S, Delgererekh B, Martineau AR. Influence of vitamin D supplementation on growth, body composition, and pubertal development among school-aged children in an area with a high prevalence of vitamin D deficiency: a randomized clinical trial. *JAMA Pediatr* 2023;**177**:e224581. doi:10.1001/jamapediatrics.2022.4581.
- [24] Sudfeld CR, Mugusi F, Muhigi A, Aboud S, Nagu TJ, Ulena N, et al. Efficacy of vitamin D3 supplementation for the prevention of pulmonary tuberculosis and mortality in HIV: a randomised, double-blind, placebo-controlled trial. *Lancet HIV* 2020;**7**:e463–71. doi:10.1016/S2352-3018(20)30108-9.
- [25] Cadranel J, Hance AJ, Milleron B, Paillard F, Akoun GM, Garabedian M. Vitamin D metabolism in tuberculosis. Production of 1,25(OH)2D3 by cells recovered by bronchoalveolar lavage and the role of this metabolite in calcium homeostasis. *Am Rev Respir Dis* 1988;**138**:984–9. doi:10.1164/ajrccm/138.4.984.
- [26] Vasilevskaya AV, Yantsevich AV, Sergeev GV, Lemish AP, Usanov SA, Gilep AA. Identification of Mycobacterium tuberculosis enzyme involved in vitamin D and 7-dehydrocholesterol metabolism. *J Steroid Biochem Mol Biol* 2017;**169**:202–9. doi:10.1016/j.jsmb.2016.05.021.
- [27] Buonsenso D, Focarelli B, Scalzone M, Chiaretti A, Gioè C, Ceccarelli M, et al. Chest wall TB and low 25-hydroxy-vitamin D levels in a 15-month-old girl. *Ital J Pediatr* 2012;**38**:12. doi:10.1186/1824-7288-38-12.
- [28] Buonsenso D, Pata D, Colonna AT, Ferrari V, Salerno G, Valentini P. Vitamin D and tuberculosis in children: a role in the prevention or treatment of the disease? *Monaldi Arch Chest Dis* 2022;**92**. doi:10.4081/monaldi.2022.2112.
- [29] Buonsenso D, Sali M, Pata D, Masiello E, Salerno G, Ceccarelli M, et al. Vitamin D levels in active TB, latent TB, non-TB pneumonia and healthy children: a prospective observational study. *Fetal Pediatr Pathol* 2018;**37**:337–47. doi:10.1080/15513815.2018.1509407.
- [30] Vieth R. The pharmacology of vitamin D, including fortification strategies. In: Glorieux FH, Pike JW, Feldman D, editors. *Vitamin D*. London: Academic Press Inc.; 2005. p. 995–1015.
- [31] Qiagen. QuantiFERON®-TB Gold Plus (QFT®-Plus) package insert, <https://www.quantiferon.com/us/wp-content/uploads/sites/13/2019/07/L1095849-R05-QFT-Plus-ELISA-IFU-USCA.pdf>; 2019 [accessed 02 December 2022].