

Vitamin D concentrations in infancy and the risk of tuberculosis in childhood:

A prospective birth cohort in Cape Town, South Africa

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Summary:

Low vitamin D may increase the risk of tuberculosis; however, previous cohort studies have had variable results. In a South African birth cohort, vitamin D levels in infancy were not associated with tuberculosis over a median of 7.2 years follow-up.

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Abstract

Introduction

Low vitamin D may increase the risk of tuberculosis; however, previous observational cohort studies have had variable results. We investigated the relationship between vitamin D levels in infancy and subsequent development of tuberculosis throughout childhood.

Methods

We enrolled pregnant women between 20–28 weeks' gestation attending antenatal care in a peri-urban South African setting in the Drakenstein Child Health Study. Serum 25(OH)D concentrations were measured in newborn infants between 6–10 weeks of age. Children were followed prospectively for tuberculosis infection and disease using annual tuberculin skin testing, radiographic examinations, and microbiological diagnosis with GeneXpert, culture, and smear testing. Univariable and multivariable Cox regression was performed and hazard ratios (HRs) with 95% confidence intervals (CIs) were calculated.

Results

Children were followed for tuberculosis for a median of 7.2 years (IQR, 6.2–7.9). Among 744 children (< 1% living with HIV, 21% HIV-exposed living without HIV), those who were vitamin D deficient in early infancy were not at increased risk of developing tuberculosis (AHR, 0.8; 95% CI, 0.4–1.6). Infants in the lowest vitamin D concentration tertile were at similar risk of tuberculosis compared to the highest tertile (AHR, 0.7; 95% CI, 0.4–1.4). Vitamin D deficiency was associated with tuberculin conversion ≤ 2 years of age at a <30nmol/l (AOR, 1.9; 95% CI, 1.2–3.2), but not <50nmol/l (AOR, 1.5; 95% CI, 0.8–2.9), cutoff.

Conclusion

In a setting with hyperendemic tuberculosis, vitamin D levels in infancy did not predict tuberculosis at any point in childhood. However, very low vitamin D levels were associated with tuberculin conversion in young children.

Key Words: tuberculosis; pediatrics; micronutrients; vitamin D.

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Introduction

Over one million children develop tuberculosis disease every year representing substantial morbidity and mortality.^{1,2} The risk of tuberculosis disease is greatest in early childhood so understanding which children are at high risk is essential to target and prioritize finite health resources.³⁻⁵ However, other than children recently exposed to a tuberculosis case^{4,6,7} or with a positive skin test,⁴ there is uncertainty about which children health services should target and how nutritional deficiencies play a role in development of pediatric tuberculosis.

Globally, undernutrition is a major contributor to tuberculosis disease^{8,9} and several studies have investigated whether there is an association between micronutrient deficiencies and tuberculosis with varied results.¹⁰⁻¹³ Of all micronutrient deficiencies, vitamin D is commonly linked to tuberculosis disease in observational studies. Tuberculosis patients often present with lower vitamin D concentrations; however, whether low vitamin D levels predict subsequent incident tuberculosis infection or disease is unclear.¹¹ Adult studies have shown conflicting data with some studies reporting an association between low vitamin D levels and prevalent (at time of tuberculosis disease diagnosis) or incident (diagnosed during follow-up) tuberculosis disease. However, others have shown no association between low vitamin D and tuberculosis disease.¹¹ No population-based studies have been performed in infants or in settings with a high burden of both tuberculosis and vitamin D deficiency.

In a birth-cohort study from South Africa, we studied the relationship between serum vitamin D levels in infancy and subsequent development of tuberculosis disease throughout childhood. We also assessed whether vitamin D concentrations were associated with tuberculin skin test conversion.

Methods

Study design and participants

The study cohort as well as the sub-cohort tested for vitamin D has been described previously.^{3,14,15} Briefly, we enrolled pregnant women between 20 and 28 weeks' gestation attending antenatal care in Paarl, a periurban setting outside of Cape Town, South Africa. Participants were recruited from two neighboring community clinics, TC Newman and Mbekweni, serving impoverished communities. Infants received the Bacillus Calmette–Guérin (BCG) vaccination at birth. All mothers accessed care in the public sector, which has a strong primary healthcare programme, including an effective mother-to-child HIV prevention and antiretroviral therapy (ART) programme. Women were followed through pregnancy and childbirth, and newborn infants were followed into childhood. Vitamin D supplementation was not routinely provided to infants in the national health services, unless born premature. Exclusion criteria for pregnant women were being younger than 18 years and intending to leave the area within 1 year.

We obtained ethics approval from the University of Cape Town Faculty of Health Sciences Human Research Ethics Committee (reference numbers 401/2009 and 651/2013) and the Provincial Child Health Research Committee. Mothers provided written informed consent at enrolment, which was renewed annually.

Procedures

Surveys focusing on maternal health were administered at enrollment and antenatal data were concurrently collected. Detailed birth information was obtained at delivery. Obstetric care and all births took place at the regional hospital in Paarl. Follow-up visits, including clinical examinations, were done at 6, 10, and 14 weeks, 6 and 12 months, and then annually until the end of follow-up. Data for environmental exposures, household characteristics, respiratory risk factors, anthropometry, and child symptoms were obtained at scheduled visits. Missed visits were rebooked with a study mobile phone network system or by study community-based fieldworkers. Mothers were counselled about respiratory symptoms at every visit and advised to attend the study site or contact study staff between scheduled study visits whenever the child developed cough or difficulty breathing.

All mothers were tested for HIV during pregnancy with Abbott Determine HIV 1/2 rapid HIV antibody test (Abbott Laboratories, North Chicago, IL, USA). If positive, a confirmatory enzyme-linked immunosorbent assay was done. All mothers living with HIV received ART as per national guidelines. Infants of mothers living with HIV were tested with DNA PCR (Cobas Ampliprep system, Roche Molecular Systems, Branchburg, NJ, USA) at age 6 weeks, and 6 weeks after the end of breastfeeding as per national guidelines. Children were re-tested at 18 months with the rapid antibody test.^{16,17}

Serum samples were taken from infants between 6–10 weeks of age. Vitamin D status was assessed through serum 25-hydroxyvitamin D (25(OH)D) concentration (nmol/L) levels and measured at Vitas AS (Oslo, Norway; a reference laboratory in Europe with a Vitamin D External Quality Assessment Scheme certificate) from specimens stored at –80°C using liquid chromatography–tandem mass spectrometry. Clinicians did not have

access to vitamin D results when making diagnoses, as measurements were done on biobanked samples several years after collection.

Data was collected on infant factors relevant to vitamin D and tuberculosis disease risk based on prior studies with this cohort^{3,15} as well as the medical literature.¹⁸ Infant variables included sex, height-for-age z score (HAZ), weight-for-age z score (WAZ), maternal HIV, gestational age, breastfeeding practices in the first year of life, and season of birth. Season of birth was categorized into summer (December–February), autumn (March–May), winter (June–August), and spring (September–November). We also collected maternal factors including age, smoking, educational level achieved, and various markers for household socio-economic status. Socioeconomic status was assessed using a validated composite score comprising four variables comprising asset ownership, household income, employment, and education.¹⁴

Tuberculin skin tests were done at the 6-month visit and then at 12, 24, 36, 48, and 60 months of age, and at the time of a lower respiratory tract infection (LRTI).³ Tuberculin skin test conversion was defined as an induration reaction greater than or equal to 10 mm, to minimize the risk of misclassification due to BCG vaccination or exposure to environmental mycobacteria.^{19,20} As tuberculin skin test boosting may occur after recurrent tests, children with reactive but negative skin test (1–9 mm) were not given another test and were censored from conversion analysis at that timepoint. Because most children were censored by two years of age in our cohort, we only used tuberculin skin test results before this age in this analysis. Children with positive tuberculin skin tests were screened for tuberculosis disease and, if none, were referred to local tuberculosis clinics for isoniazid preventive therapy.

Children were followed up for tuberculosis disease from birth at regular study visits as previously described.³ Tuberculosis disease was diagnosed by experienced physicians and nurses in local tuberculosis community clinics or by study staff, and chest radiographs

were read and reported by an experienced clinician. Trained staff collected induced sputum for microbiological confirmation using liquid culture and nucleic acid amplification (Xpert MTB/RIF; Cepheid, Sunnyvale, CA, USA) from children with a tuberculin skin test induration ≥ 10 mm, those presenting with a LRTI, and in participants in whom tuberculosis disease was considered presumptive. A chest radiograph was taken in all children with presumptive (or possible) tuberculosis disease.

Statistical analysis

Children were included in this analysis if the child had a vitamin D measurement at 6–10 weeks of age. We summarized continuous variables as medians with interquartile ranges (IQR) and categorical variables using proportions.

Our primary outcome was tuberculosis disease incidence after 10 weeks of age to the end of follow-up (at January 30, 2021). For tuberculosis disease incidence, time-to-event was constructed between birth and the date of tuberculosis. Follow-up was censored at death, development of tuberculosis disease, end of follow-up, or until January 30, 2021. We compared tuberculosis disease incidence in infants with and without vitamin D deficiency using hazard ratios (HRs) and 95% confidence intervals (CIs) obtained from Cox proportional hazards models. We completed this analysis independently for the entire follow-up and then conducted a landmark analysis (i.e., analyzing only subjects at that time point still eligible for the analysis) prior to 1 year of age. Two-sample likelihood ratio tests were used.

To assess whether there was a dose-response relationship between vitamin D concentration, we categorized vitamin D concentrations into tertiles, as used elsewhere and to enable comparison with such studies.^{11,12,15} We compared incident tuberculosis disease among participants at each tertile level. We also compared our results using vitamin D cutoffs. Children were categorized

into distinct categories based on their serum 25(OH)D concentration level including deficient (<50 nmol/l), insufficient (50–74 nmol/l), and sufficient (\geq 75 nmol/l). There is no consensus on the definition of vitamin D deficiency.¹⁸ Due to this, we conducted separate analyses with <50 nmol/L and <30 nmol/L cut-offs for vitamin D deficiency in our cohort.¹⁸

Lastly, we assessed the relationship between vitamin D levels and tuberculin conversion \leq 1 and 2 years of age using logistic regression models. We assessed the odds of a tuberculin conversion \leq 1 or 2 years of age independently for each vitamin D deficiency cutoff and in each vitamin D tertile. Multivariable models were built including all relevant variables related to tuberculin conversion in this cohort.^{3,15} All analyses were performed using Stata (version 14.1).

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Results

Pregnant women were recruited and enrolled between March 5, 2012, and March 31, 2015, with 1,225 women included in the birth cohort (Figure 1). Of 1,143 live births, 30 (3%) were excluded because of perinatal death or study termination and 339 infants were enrolled but did not have a valid test for vitamin D. In total, 774 (2 [$<1\%$] living with HIV, 166 [21%] HIV-exposed living without HIV) children were tested for vitamin D and included in our analysis (Table 1). The median WAZ and HAZ scores at the time of the vitamin D measurement was -0.61 (IQR, -1.32, 0.04) and 0.01 (IQR, -0.86, 0.87).

The median vitamin D concentration was 37.4 nmol/l (IQR, 25.8–47.3). The number of infants classified as vitamin D deficient at cutoffs of <50 nmol/l and <30 nmol/l were 624 (81%, 95% CI, 78–83) and 242 (31%, 95% CI, 28–35), respectively. Almost half of the cohort ($n=382$, 49%) had vitamin D levels between 30–50 nmol/l. Only 1% of infants were vitamin D sufficient at a cutoff of ≥ 75 nmol/l.

Children were followed for tuberculosis disease for a median of 7.2 years (IQR, 6.2–7.9). Over 5,167 child-years of follow-up, 62 children were diagnosed with tuberculosis disease (1,123 cases per 100 thousand person-years, 95% CI, 868–1,452). One (1.4%) case manifested as disseminated tuberculosis; the remaining 61 cases were diagnosed with pulmonary tuberculosis. Among all cases, 12 (19.7%) were microbiologically confirmed.

There was no observed statistical relationship between vitamin D levels in early infancy and tuberculosis disease incidence during childhood regardless of the cutoff used (Table 2 and S1; Figure 2). The incidence of tuberculosis disease per 100 thousand child-years was not statistically higher among infants that were and were not vitamin D deficient at a cutoff <50 nmol/l (1,137 [95% CI, 854–1,513] versus 1,066 [95% CI, 591–1,926]). The

incidence of tuberculosis disease was similar when using an alternative cutoff of <30 nmol/l (1,202 [95% CI, 891–1,620]).

In a multivariable model adjusting for maternal HIV, breastfeeding, sex, socioeconomic status, and study site, the hazard of tuberculosis disease was statistically similar among children with and without vitamin deficiency when defining vitamin D deficiency at either <50 nmol/l (AHR, 0.8; 95% CI, 0.4–1.6) or <30 nmol/l (AHR, 1.5; 95% CI, 0.7–3.1). When adding tuberculin skin test conversion ≤ 2 years of age to this model, the hazard of tuberculosis did not alter substantially at either <50 nmol/l (AHR, 1.0; 95% CI, 0.5–2.2) or <30 nmol/l (AHR, 1.0; 95% CI, 0.5–1.8), respectively. This null relationship did not change when we restricted follow-up time or controlled for additional potential confounders (Table S1). There was a consistent null relationship between vitamin D deficiency and incidence of tuberculosis disease when we restricted follow-up to before 12 months of life (<50 nmol/l, AHR, 0.7; 95% CI, 0.3–1.7; <30 nmol/l, AHR, 1.5; 95% CI, 0.7–3.1). Similarly, there was no statistical relationship between vitamin D tertiles and incident tuberculosis disease ($P_{trend}=0.5607$). Compared to the highest vitamin D tertile, children in the lowest tertile had similar risk of developing incident tuberculosis disease (AOR, 0.8, 95% CI, 0.3–1.9) (Figure 2). In multivariable linear regression models, we found no relationship between vitamin D concentrations in infancy and subsequent risk of tuberculosis disease over follow-up (0.4 nmol/L; 95% CI, -0.2, 1.0) or during the first year of life (-1.0 nmol/L; 95% CI, -6.7, 4.6).

In a multivariable logistic regression model adjusting for study site, season of birth, household tuberculosis exposure, breastfeeding, sex, and maternal HIV, vitamin D deficiency in infancy was associated with tuberculin conversion ≤ 2 years of age at a cutoff of <30 nmol/L (AOR, 1.9, 95% CI, 1.2–3.2) but not <50 nmol/L (AOR, 1.5; 95% CI, 0.8–2.8). Lower vitamin D tertiles were associated with tuberculin conversion ≤ 1 ($P_{trend}=0.0048$) and 2 years of age ($P_{trend}=0.0083$). Compared to the highest tertile, children in the lowest vitamin D tertile had over 2 times greater odds of tuberculin conversion (AOR, 2.7, 95% CI, 1.4–5.3) and ≤ 2 years old (AOR, 2.3, 95% CI, 1.2–4.3). Children in

the middle tertile were at non-statistically greater odds of tuberculin conversion ≤ 1 (AOR, 1.9, 95% CI, 1.0–3.8) and 2 years old (AOR, 1.6, 95% CI, 0.8–2.9) (Figure 3).

Discussion

In this prospective, population-based birth cohort study in South Africa, we found no relationship between vitamin D levels in early infancy and incident tuberculosis disease throughout childhood. This persisted after controlling for a range of potential confounders including socioeconomic status and tuberculosis exposure as well as when restricting follow-up time and varying cutoffs for vitamin D deficiency. However, children with the lowest vitamin D levels in early infancy were more likely to convert their tuberculin skin test in the first 2 years of life. In settings with hyperendemic tuberculosis disease and where vitamin D deficiency is ubiquitous, such as South Africa, vitamin D concentrations in infancy may not predict subsequent tuberculosis disease in childhood.

Our results suggesting no relationship between vitamin D and incident tuberculosis disease conflict with some, but not all, prior observational studies.^{11,20,21} Several observations may explain these differences. First, almost all previous studies were among groups at high-risk of tuberculosis disease, such as household contacts of tuberculosis cases or persons living with HIV. A recent individual-participant meta-analysis found a dose-response relationship between vitamin D levels and incident tuberculosis disease¹¹, predominantly driven by persons living with HIV.²⁰ In a household contact study from Peru however, no relationship between vitamin D and incident tuberculosis disease was found.¹¹ Our study is among the first population-based studies to investigate this question. Second, we only measured vitamin D levels in infants; previous studies included predominantly adults. Our study extends prior results to young children. Vitamin D levels

in young children, especially in studies from sub-Saharan Africa,¹⁸ indicate that vitamin D levels may be lowest at this young age and this may modify our results.¹⁸ Lastly, most previous studies have been limited by short follow-up, typically in the range of 1–2 years.¹¹

Our null finding may be partially explained by the extraordinarily high prevalence of vitamin D deficiency at the most used cutoff, <50 nmol/l.^{11,18} Over 80% of infants in our study were vitamin D deficient at this cutoff and only 1% of infants were vitamin D sufficient. Our findings may be distinct from results from other settings where vitamin D deficiency is less common.^{18,23} A systematic review of vitamin D prevalence levels in Africa found wide heterogeneity by age, location, and deficiency cutoffs.¹⁸ Among few studies of newborn infants in Africa, the prevalence of vitamin D deficiency ranged from 9% in Tanzania to 98% in Tunisia (at a <50 nmol/l cutoff). It's unclear if a relationship between vitamin D and incident tuberculosis disease is modified by the background burden of vitamin D deficiency or tuberculosis disease.

Our results of an association between vitamin D levels and tuberculin skin test conversion at 1 and 2 years of life was largely dependent on the vitamin D cutoff used. Using a more inclusive definition of <50 nmol/l, we found no statistical association between vitamin D status and tuberculin conversion. With a more stringent <30 nmol/l cutoff, we found a positive association. The distinct results using different cutoffs in our study is not surprising given that approximately 50% of our cohort had vitamin D concentrations between 30–50 nmol/l. Our results suggest that children may be at particularly high risk to convert their tuberculin skin test if their vitamin D levels are especially low. The distribution of vitamin levels should be considered rather than standardized cutoffs when investigating the relationship between tuberculosis and micronutrients. A recent clinical trial of vitamin D supplementation among 6- to 13-year-old children found no impact on QuantiFERON conversion after 3 years of follow-up.²⁴ It's unclear if our study provides elucidation on these trial results as there are substantial

setting and study design differences in participant ages, baseline vitamin D levels, the background force of tuberculosis infection, and the use of diagnostic test for conversion.

There are several strengths of our study. We prospectively followed-up participants through childhood. In addition, the community-based sample allowed us to understand the degree of exposure to vitamin D deficiency in this population. These results may not be generalizable to settings with a low burden of tuberculosis disease. However, the prevalence of tuberculosis disease and vitamin D deficiency is high in many African and low-income countries; furthermore, the inclusion of two distinct communities, with risk factors such as poor nutrition, HIV exposure or poverty are likely to make these results generalizable to similar communities. Lastly, surveillance for tuberculosis disease included a wide range of tests such as tuberculin skin tests, chest radiographs, smear and culture, and Xpert MTB/RIF. Children in our cohort were also assessed for other diseases which may increase tuberculosis case detection. Our study also has limitations. An important limitation was that we only measured vitamin D levels at 6–10 weeks of age; micronutrient levels are not constant and fluctuate throughout early childhood. For example, in the United States, a high-income, low tuberculosis burden setting, there were wide fluctuations in vitamin D concentration from infancy to early childhood and transient vitamin D deficiency was common.²³ How vitamin D concentrations fluctuate over time in young children from South Africa is unknown but is likely to be distinct from children from the United States and other high-income countries. It is also unclear whether vitamin D trajectories, rather than a vitamin D measurement at one point in time, provide additional prognostic value for incident tuberculosis disease. Maternal vitamin D status and vitamin supplementation during pregnancy, potential modifiers of infant vitamin D levels, were not assessed. In addition, boosting through BCG vaccination or repeated tuberculin skin tests may lead to false-positive conversion results. To attempt to address this, any child with a positive skin test reaction of any size did not have a repeat skin test, potentially impacting our conversion results. The use of interferon-gamma assays may have limited bias through BCG boosting, but the need for a blood sample, lab testing, and cost, are not likely feasible in low- and middle-income

settings.²⁵ We attempted to minimize this bias by using a conservative skin test conversion cutoff (10mm induration) for this population.²⁶

In conclusion, in a setting with hyperendemic tuberculosis disease and where vitamin D deficiency is ubiquitous, vitamin D levels in infancy did not predict subsequent tuberculosis disease at any point in childhood. Whether children with vitamin D deficiency had greater risk of converting their tuberculin skin test depended on the cutoff definition used for vitamin D deficiency.

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NOTES:

Contributors

LM analysed the data and wrote the first draft of the manuscript. HJZ is the principal investigator, obtained funding, conceived and designed the study, and assisted with drafting of the manuscript. MPN is the lead microbiologist. All author contributed to interpretation of results. All authors reviewed, contributed to, and approved drafts of the manuscript as well as the final manuscript.

Acknowledgments

We thank the study staff, the clinical and administrative staff of the Western Cape Government Health Department at Paarl Hospital and at the clinics for support of the study, members of the study International Advisory Board for their advice, our collaborators, and the families and children who participated in the study.

Funding

This study was funded by the Bill & Melinda Gates Foundation (grant number OPP 1017641), Medical Research Council South Africa, and the National Research Foundation South Africa. M.P.N. is supported by an Australian National Health and Medical Research Council Investigator Grant (APP1174455).

Declaration of interests

HJZ and MPN report grants from the Bill & Melinda Gates Foundation. HJZ also reports grants from Medical Research Council South Africa, the National Research Foundation South Africa, and the National Institutes of Health during completion of the study. All other authors declare no competing interests.

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Table 1. Sociodemographic and clinical characteristics of 774 mother–infant pairs.

Variables	Total (%)	Median (IQR)
Maternal characteristics		
Median Age, years (IQR)	...	26 (22 – 31)
Age group, years		
<20	89 (11.5)	
20-24	253 (32.7)	
25-29	209 (27.0)	
≥30	223 (28.8)	
Education		
Primary	58 (7.5)	
Some Secondary	413 (53.4)	
Completed Secondary	256 (33.1)	
Some Tertiary	47 (6.1)	
HIV Positive Status	166 (21.4)	
Maternal smoking in pregnancy	182 (23.5)	

Infant characteristics

Birthweight, grams		3,100 (2,750, 3,420)
Weight-for-age z score		-0.39 (-1.15, 0.33)
Height-for-age z score		-0.83 (-1.75, -0.01)
Female	366 (47.3)	
Gestation delivery, weeks		39 (38, 40)
Prematurity (< 37 weeks)	98 (12.7)	
Breastfeeding initiated	718 (92.8)	
HIV Positive Status	2 (0.3)	
Season birth		
Summer (Dec–Feb)	220 (28.4)	
Autumn (March–May)	195 (25.2)	
Winter (June–August)	189 (24.4)	
Spring (Sept– Nov)	170 (22.0)	

Household Characteristics

Socioeconomic Status		
Lowest	190 (23.8)	
Moderate Low	190 (25.2)	
Moderate high	195 (25.6)	

High

193 (25.4)

Abbreviations. yo, years old. IQR, interquartile range. HIV, human immunodeficiency virus.

*Percentages refer to within-characteristic column totals among participants within each clinic and in entire study. Percentages may not total 100% because within-column percentages were rounded to the nearest integer. Column totals vary across different characteristics due to missing values for some participants.

†We derived Z scores from World Health Organization child growth standards at birth and at every follow-up visit; we used the median of all the weight-for-age Z scores for each child to summarize nutrition status over the duration of follow-up.

‡Socioeconomic status comprised a comprehensive composite of asset ownership, household income, employment, and education.

Table 2. Association between vitamin D concentrations and risk of incident tuberculosis disease.

	Median (IQR)	Person-years	Incident tuberculosis disease*	Univariable model (HR, 95% CI)	Multivariable model (AHR, 95% CI)
All follow-up					
Vit D deficient, <50 nmol/l	33.9 (22.4, 41.4)	4146.7	51	1.1 (0.6-2.1)	0.8 (0.4-1.6)
Vit D deficient, <30 nmol/l	17.3 (9.0, 24.3)	1595.6	26	1.3 (0.8-2.3)	1.5 (0.7-3.1)
Vitamin D concentration					
Tertile 1 (n = 258)	18.6 (9.8, 25.8)	1706.8	27	1 (referent)	1 (referent)
Tertile 2 (n = 258)	37.4 (34.3, 41.0)	1698.1	19	0.7 (0.4-1.3)	0.8 (0.5-1.5)
Tertile 3 (n = 258)	51.4 (47.3, 58.8)	1773.3	16	0.6 (0.3-1.1)	0.7 (0.4-1.4)
<i>P_{trend}</i>				0.083	0.4229
<1 year of age					
Vit D deficient, <50 nmol/l	33.9 (22.4, 41.5)	624.0	22	0.9 (0.4-2.1)	0.7 (0.3-1.7)
Vit D deficient, <30 nmol/l	17.3 (9.0, 24.3)	242.0	13	1.3 (0.6-2.5)	1.5 (0.7-3.1)
Vitamin D concentration					

Tertile 1 (n = 258)	18.6 (9.8, 25.8)	250.4	16	1 (referent)	1 (referent)
Tertile 2 (n = 258)	37.4 (34.2, 41.0)	253.8	10	0.6 (0.3-1.4)	0.8 (0.4-1.8)
Tertile 3 (n = 258)	51.4 (47.3, 58.8)	254.6	8	0.5 (0.2-1.1)	0.8 (0.3-1.9)
<i>P_{trend}</i>				0.101	0.5607

* This is the number of incident tuberculosis cases in the specified row but may not include all cases in the comparison group. For example, in the first row describing the ‘Vit D deficient, <50 nmol/l’ group the number of incident tuberculosis disease cases is 16 but the number of cases in the vitamin D insufficient/sufficient comparator group is not listed.

† All models are adjusted for sex of the child, study site, season of birth, and maternal HIV using cox regression models.

‡ Follow-up time was restricted to certain ages based on distance from birth. The specified time indicates the starting point time. For example, the primary outcome is follow-up for tuberculosis disease starting at 1 year of age until the end of follow-up.

Figure Legends

Figure 1. Study flow diagram of eligibility and enrollment of mothers and infants in the Drakenstein Child Health study, Cape Town, South Africa.

*Loss of pregnancy due to miscarriage, stillbirth, or intrauterine death (23 infants [including one set of twins]).

† Including four pairs of twins and one set of triplets.

‡ No postnatal data collected.

Figure 2. Low vitamin D concentrations or vitamin D deficiency and the risk of subsequently developing tuberculosis disease throughout childhood.

Models in both panels are adjusted for child sex, maternal HIV status, and study site.

Figure 3. Vitamin D concentrations and the odds of tuberculin skin test conversion in the first 2 years of life.

† All models are adjusted for sex of the child, household tuberculosis exposure, breastfeeding, study site, and maternal HIV.

Figure 1

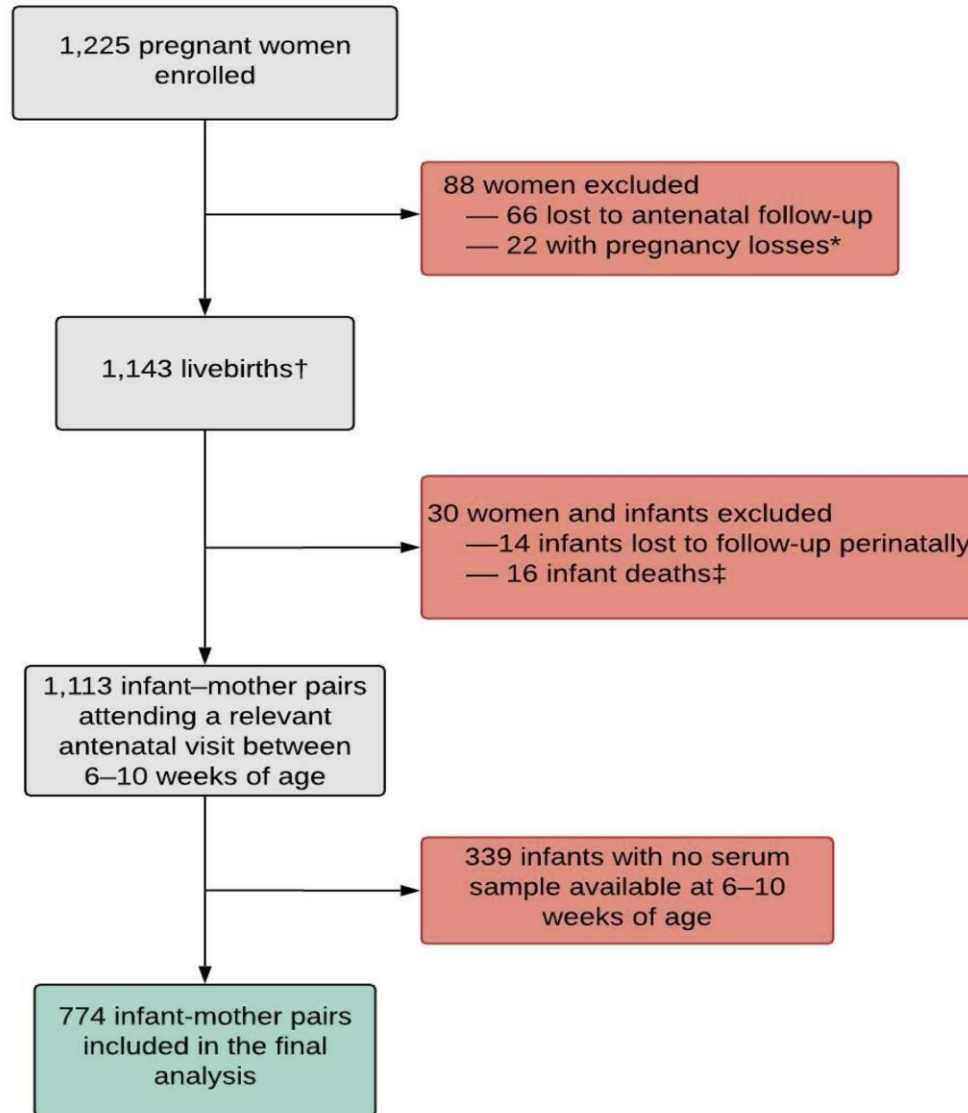


Figure 2

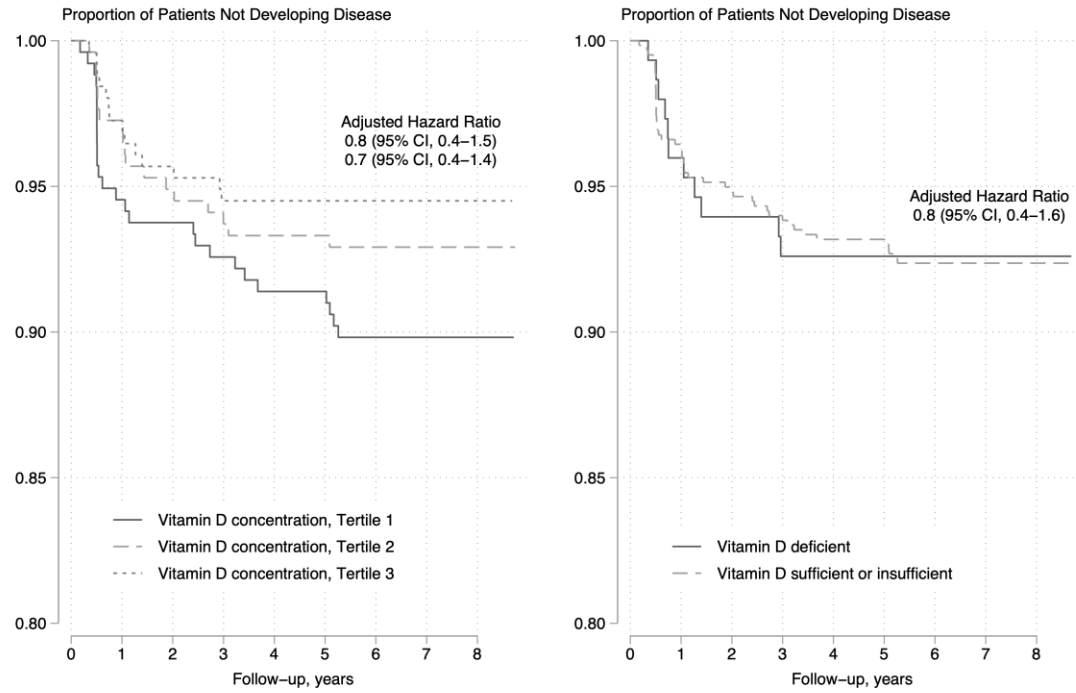
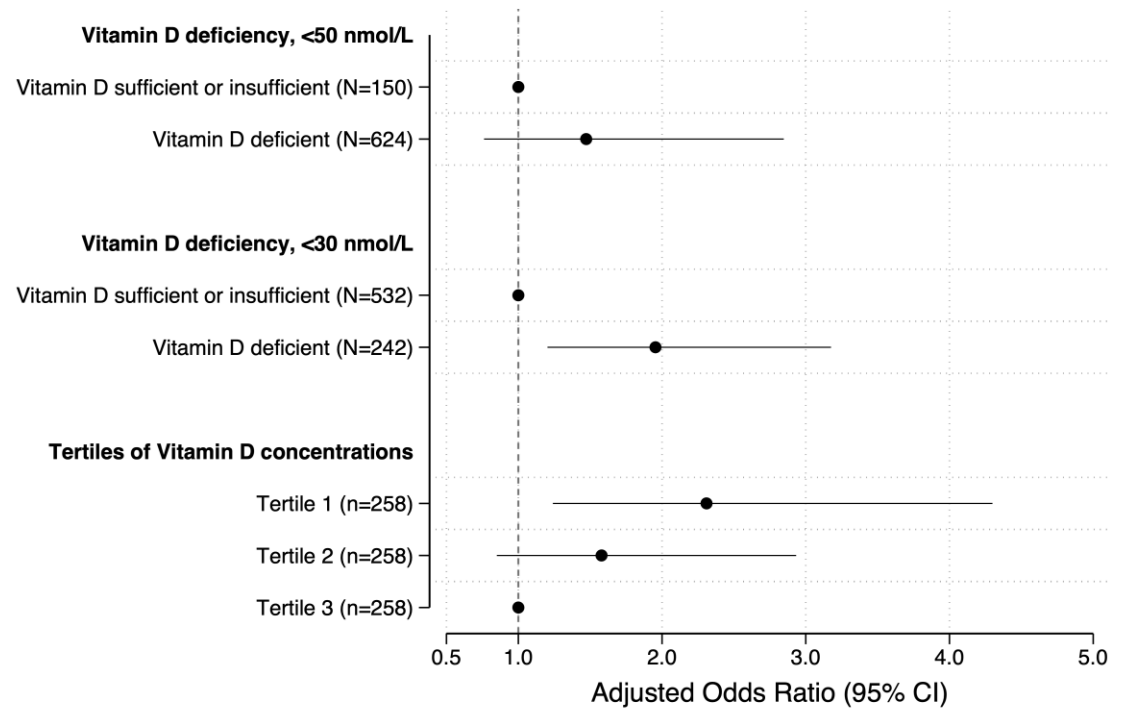


Figure 3



ACCEPTED