1 **Title – The Impact of Vitamin A and Carotenoids on the Risk of Tuberculosis Progression**

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24   **Abstract**

25   **Background:** Low and deficient levels of vitamin A are common in low and middle income

26   countries where tuberculosis burden is high. We assessed the impact of baseline levels of

27   vitamins A and carotenoids on TB disease risk.

28   **Methods and Findings:** We conducted a case-control study nested within a longitudinal cohort

29   of household contacts of pulmonary TB cases in Lima, Peru. We screened all contacts for TB

30   disease at 2, 6, and 12 months after enrollment. We defined cases as HIV-negative household

31   contacts with blood samples who developed TB disease at least 15 days after enrollment of the

32   index patient. For each case, we randomly selected 4 controls from among contacts who did not

33   develop TB disease, matching on gender and year of age. We used conditional logistic regression

34   to estimate odds ratios (ORs) for incident TB disease by vitamin A and carotenoids levels,

35   controlling for other nutritional and socioeconomic factors.

36   Among 6751 HIV-negative household contacts with baseline blood samples, 192

37   developed secondary TB disease during follow-up. We analyzed 180 cases with viable samples

38   and 709 matched controls. After controlling for possible confounders, we found that baseline

39   vitamin A deficiency was associated with a 10-fold increase in risk of TB disease among

40   household contacts (aOR 10.42; 95% CI 4.01–27.05; $p < 0.001$). This association was dose-

41   dependent with stepwise increases in TB disease risk with each decreasing quartile of vitamin A

42   level. Carotenoid levels were also inversely associated with TB risk among adolescents.

43   Our study is limited by the one year duration of follow up and by the relatively few blood

44   samples available from household contacts under ten years of age.

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45   **Conclusions:** Vitamin A deficiency strongly predicted risk of incident TB disease among

46   household contacts of TB patients. Vitamin A supplementation among individuals at high risk of

47   TB may provide an effective means of preventing TB disease.

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Introduction

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74   Although tuberculosis (TB) incidence and mortality have fallen steadily over the past 150

75   years in Europe and North America, TB remains one of the top global causes of mortality from

76   an infectious disease. The World Health Organization (WHO) reported 10.4 million new cases

77   and 1.8 million TB deaths in 2015, the vast majority of which occurred in low- and middle-

78   income countries [1]. In high-income countries, TB declined prior to the availability of either

79   Bacillus Calmette–Guérin (BCG) vaccine or effective chemotherapy, and observers attribute this

80   trend to improvements in socioeconomic status (SES) [2]. Country-level data support this

81   hypothesis; Janssens and Rieder found an inverse linear association between per capita GDP and

82   TB incidence [3], and Dye et al. noted that a nation's human development index strongly

83   predicted changes in TB incidence over time [4].

84   Multiple lines of evidence suggest that the association between SES and TB may be

85   mediated through nutritional status. Studies in both humans and animals demonstrate a link

86   between undernutrition and TB [5]. *In vitro* studies also provide insight into specific anti-

87   mycobacterial mechanisms mediated by micronutrients [6,7]. Although multiple studies

88   document micronutrient deficiencies in people with TB disease [8–10], few prospective studies

89   have assessed preexisting nutritional status as a determinant of progression from TB infection to

90   disease [11–15]. Notably, studies of vitamin A and TB have generally been conducted in

91   populations at low risk for both nutritional deficits and TB and have been inconclusive [14,15].

92   Despite the lack of data on TB risk, multiple previous studies support the role of vitamin

93   A in both innate and acquired immune responses to infection. Based on a series of studies they

94   conducted in both animals and humans, Mellanby and Green first proposed that vitamin A is an

95   "anti-infective" agent in the 1920s [16–18]. Subsequently, in one of the earliest reported clinical

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96 trials, Ellison showed that vitamin A supplementation reduced mortality in children with measles

97 by 58% [19]. Current evidence demonstrates that vitamin A deficiency contributes to mortality

98 from diarrheal diseases and measles, accounting for 1.7% of all deaths in children younger than

99 five in low and middle income countries [20].

100   Here, we assessed the association between baseline levels of vitamin A and carotenoids

101 and the risk of TB disease among household contacts of patients with pulmonary TB in Lima,

102 Peru.

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104 **Methods**

105   The study was approved by the Institutional Review Board of Harvard School of Public

106 Health and the Research Ethics Committee of the National Institute of Health of Peru. All

107 participants or guardians provided written informed consent.

108 **Setting and Study Design**

109   We conducted a case-control study nested within a prospective longitudinal cohort of

110 household contacts (HHCs) of TB cases in Lima, Peru. The study area includes 20 districts of

111 metropolitan Lima with a population of approximately 3.3 million residents living in urban areas

112 and peri-urban, informal shantytown settlements. Between September 2009 and August 2012, we

113 identified index patients 15 years and older diagnosed with pulmonary TB at 106 participating

114 health centers. Within two weeks of enrolling an index patient, we enrolled his or her HHCs and

115 screened for TB disease. HHCs with symptoms were referred for sputum smear microscopy and

116 mycobacteriology culture and treatment according to Peru's national guidelines [21]. HHCs aged

117 19 and under, and individuals with specified co-morbidities were offered isoniazid preventive

118 therapy (IPT).

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119   Upon enrollment of HHCs, we collected the following: age, gender, height, weight, BCG

120   vaccination scars, IPT use, alcohol and tobacco use, self-reported diabetes mellitus (DM),

121   comorbid diseases (heart disease, high blood pressure, asthma, kidney disease, use of steroids or

122   chemotherapy or immunosuppressant, any other self-reported chronic illness), TB disease

123   history, and housing asset information (housing type, number of rooms, water supply, sanitation

124   facilities, lighting, composition of exterior walls and floor and roof materials). In HHCs with no

125   prior history of TB infection or disease, we obtained a tuberculin skin test (TST). We offered

126   HIV testing to HHCs and invited all HHCs to provide a venous blood sample; 60% of those ≥10

127   years old provided this sample.

128   All HHCs were evaluated for signs and symptoms of pulmonary and extra-pulmonary TB

129   (EPTB) disease at two, six, and 12 months after enrollment. We considered HHCs to have

130   developed incident secondary TB disease if the diagnosis was confirmed by a health center

131   physician more than 14 days after index case enrollment and with co-prevalent disease if they

132   were diagnosed earlier. We defined secondary TB disease in HHCs under age 18 according to

133   consensus guidelines for diagnosis of TB disease in children [22].

134   For this study, we defined "cases" to be HIV-negative HHCs who developed incident

135   secondary TB disease during one year of follow-up. We randomly selected four controls for each

136   case from among HHCs who did not develop TB disease within one year, matching on gender

137   and age by year.

138   **Laboratory Methods**

139   We stored serum samples at –80°C after collection until analysis at end of study follow-

140   up. Laboratory personnel were unaware of the specimens' case-control status. All samples were

141   handled identically and assayed randomly. Levels of retinol and carotenoids (lutein+zeaxanthin,

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142   β-cryptoxanthin, total lycopene, α-carotene, and total β-carotene) were measured by high-

143   performance liquid chromatography [23]. The interassay coefficients of variation for retinol and

144   carotenoids were < 5%. We also measured levels of total 25–(OH)D with a commercial

145   competitive enzyme immunoassay kit (Immunodiagnostic Systems Inc., Fountain Hills, AZ).

146   The coefficient of variation at different levels of 25–(OH)D ranged from 4.6% to 8.7%.

Statistical Analysis

148   We defined vitamin A deficiency (VAD) as serum retinol < 200 µg/L (0.70 µmol/L) [24]

149   and vitamin D deficiency as serum 25–(OH)D < 50 nmol/L, insufficiency as 50–75 nmol/L and

150   sufficiency as > 75nmol/L [11,25]. We calculated body mass index (BMI) as weight in kilograms

151   divided by square of height in meters. For children and adolescent HHCs < 20 years, we used

152   WHO age and gender-specific BMI z-scores tables to classify those with BMI z-score < –2 as

153   underweight and those with z-score >2 as overweight [26]. For adults ≥ 20 years, we classified

154   nutritional status as: underweight (BMI < 18.5 kg/m^2), normal (BMI 18.5–< 25 kg/m^2), and

155   overweight (BMI ≥ 25 kg/m^2). We classified HHCs as heavy drinkers if they drank ≥ 40g or ≥ 3

156   alcoholic drinks daily based on past year recall. We derived an SES score using principal

157   components analysis of housing asset index weighted by household size [27]. We considered

158   HHCs infected with TB at baseline if they reported previous TB disease or a positive TST or had

159   a TST ≥ 10 mm at enrollment.

160   We constructed conditional logistic regression models to evaluate the association

161   between quartiles of vitamin A and carotenoids and risk of TB disease. We performed tests for

162   linear trend across quartiles. Multivariable-adjusted models included baseline covariates

163   identified a priori as potential confounders (BMI, SES, alcohol and tobacco use, receipt of IPT,

164   TB disease history, comorbid disease, self-reported DM and index patient smear status). We also

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165 assessed the impact of vitamin A deficiency on incident TB disease and examined the interaction

166 between vitamin A and D deficiency on TB disease risk. We stratified the final adjusted models

167 by age. Under our method of control selection, odds ratios approximate risk ratios because

168 secondary TB disease was relatively rare in this cohort [28].

169 In sensitivity analyses, we stratified by baseline TB infection status and separately

170 restricted the analysis to cases (and their matched controls) diagnosed at least 90 days after index

171 patient enrollment. Finally, we repeated our analyses for cases (and their matched controls) with

172 microbiologically confirmed TB.

173 For all tests, we considered two-sided p values < .05 as statistically significant. Data were

174 analyzed using SAS v9.4 (SAS Institute, 2013).

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176 **Results**

177 Among 6751 HIV-negative HHCs with blood samples, 258 HHCs developed TB disease,

178 of which 66 were co-prevalent and 192 were secondary. Pulmonary disease accounted for 236

179 (91.5%) cases and extra-pulmonary for 16 (6.2%). Of 180 secondary TB cases with viable blood

180 samples, 147 (81.7%) were microbiologically confirmed (Fig1). Table 1 shows baseline

181 characteristics of cases and controls. Cases had lower baseline median levels of vitamin A and

182 carotenoids compared to controls (Table 2). Although the pro-vitamin A carotenoids, β-

183 cryptoxanthin, α-carotene, and β-carotene contribute to retinol levels, here we found they were

184 not correlated with measured retinol levels (R 0.04 to 0.10). Table 3 demonstrates significant

185 univariate associations between incident TB disease and baseline quartiles of vitamin A,

186 lutein/zeaxanthin, β-cryptoxanthin, total lycopene, and α-carotene. After multivariable

187 adjustment for matching factors and confounders, household contacts in the lowest quartile of

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188   vitamin A had seven times the risk of TB disease compared to those in the highest quartile (OR

189   7.03; 95% CI 3.14–15.71; P for trend <0.001) [Table 3]. Lower levels of lutein/zeaxanthin (Q1

190   vs Q4: OR 2.10; 95% CI 1.17–3.77; P for trend = 0.06) and β-cryptoxanthin (Q1 vs Q4: OR

191   2.61; 95% CI 1.41–4.86; P for trend = 0.002) were also associated with increased risk of TB

192   disease.

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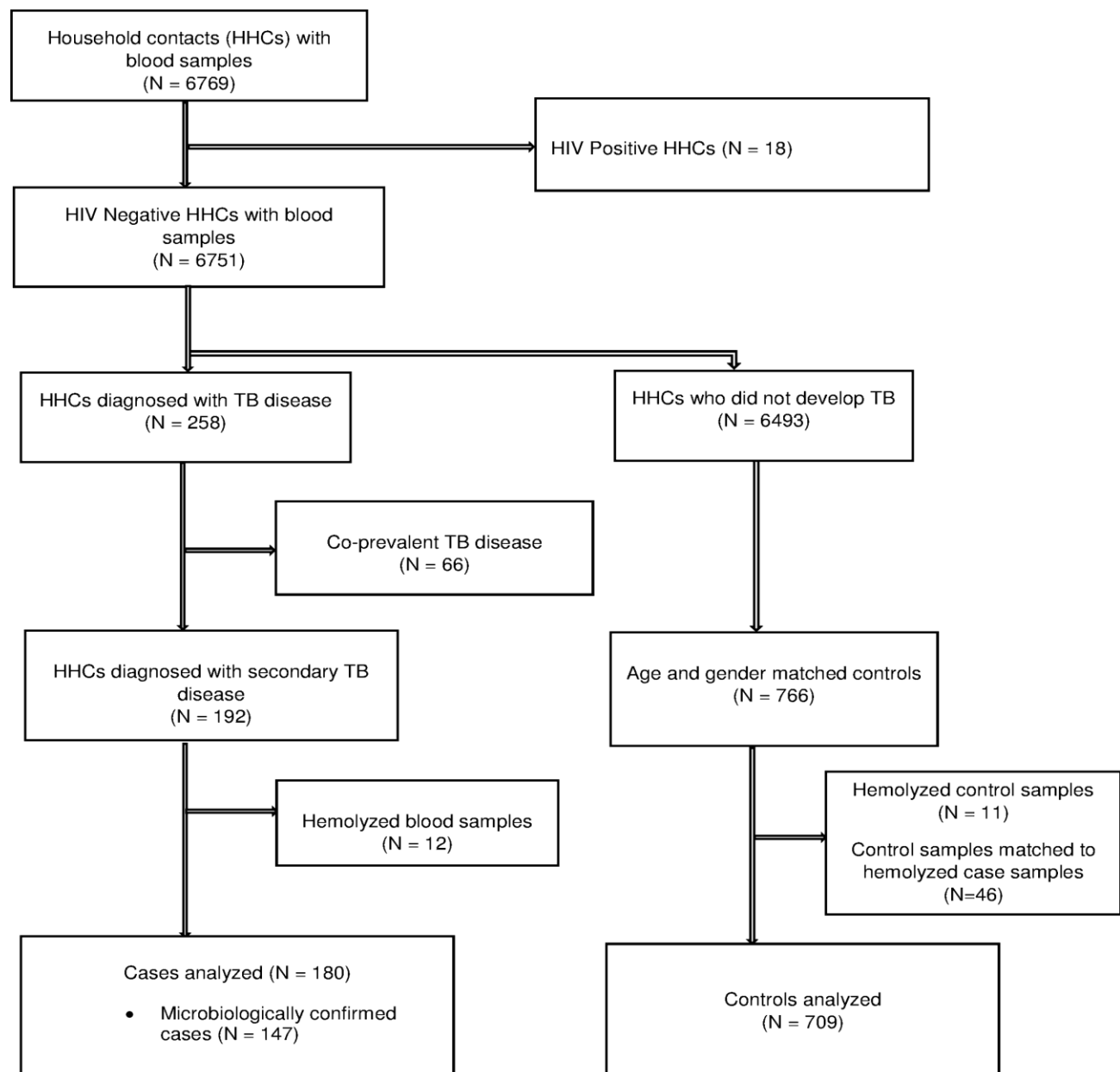
194   **Fig 1. Flow Diagram for Nested Case-Control Study**

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Table 1. Baseline Characteristics of Incident TB Cases and Matched Controls.

	Cases (N = 180) n (%)	N[a] (Cases)	Control (N = 709) n (%)	N[a] (Controls)
Age Categories in years				
< 10	4 (2.2)		16 (2.3)	
10 to 19	50 (27.8)		200 (28.2)	
≥ 20	126 (70.0)		493 (69.5)	
Male	94 (52.2)		366 (51.6)	
BMI Categories		179		706
Underweight	8 (4.5)		6 (0.9)	
Overweight	45 (25.1)		299 (42.4)	
Normal	126 (70.4)		401 (56.8)	
Socioeconomic Status		171		698
Low	77 (45.0)		228 (32.7)	
Middle	66 (38.6)		326 (46.7)	
High	28 (16.4)		144 (20.6)	
Heavy Alcohol Use	14 (8.1)	174	64 (9.3)	692
Current Smoking	13 (7.4)	176	78 (11.2)	699
Self-Reported Diabetes	6 (3.4)	179	11 (1.6)	701
Comorbid Disease	37 (20.6)		175 (24.7)	
Isoniazid Preventive Therapy	7 (3.9)		108 (15.2)	
BCG scar	159 (88.3)		628 (88.6)	
History of TB	34 (18.9)		55 (7.8)	708
TB Infection at baseline	145 (82.4)	176	281 (41.1)	683
Index Patient Characteristics				
Smear Positive	156 (86.7)		486 (68.7)	707
Cavitary Disease	54 (30.2)	179	175 (25.1)	697

BCG, Bacillus Calmette–Guérin.

[a]Total number of subjects with data for corresponding variable.

206 **Table 2. Baseline Levels of Micronutrients Among Cases and Controls.**

	Cases (N = 180) median (IQR) or n (%)	Control (N = 709) median (IQR) or n (%)
Vitamin A (µg/L)	316.5 (250.5 – 381.4)	373.6 (310.4 – 460.2)
Vitamin A Deficient (< 200 µg/L)	24 (13.3)	16 (2.3)
25–OH Vitamin D (nmol/L)	53.9 (42.7 – 64.0)	54.7 (44.5 – 67.1)
Vitamin D Deficient (< 50 nmol/L)	76 (42.2)	259 (36.5)
Vitamin D Insufficient (50–75 nmol/L)	84 (46.7)	348 (49.1)
Vitamin D Sufficient (> 75 nmol/L)	20 (11.1)	102 (14.4)
Lutein + Zeaxanthin (µg/L)	150.5 (111.1 – 186.1)	153.6 (121.5 – 201.8)
β–cryptoxanthin (µg/L)	107.3 (68.2 – 196.7)	142.3 (85.5 – 240.5)
Total Lycopene (µg/L)	99.7 (64.9 – 147.3)	112.9 (77.6 – 164.5)
α–carotene (µg/L)	65.3 (44.2 – 97.1)	75.8 (52.6 – 110.0)
Total–β–carotene (µg/L)	123.2 (67.9 – 192.4)	138.0 (86.3 – 216.4)

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209 **Table 3. Micronutrient Levels by Quartile and the Risk of TB Disease among Household**
210 **Contacts of Index TB Patients (N = 889).**

	Quartiles of micronutrients				P value for Trend
	Q1 (lowest)	Q2	Q3	Q4 (highest)	
Vitamin A					
Cases/Controls, n	76/146	45/177	41/182	18/204	
Median (Range), µg/L	258.0 (53.1 – 296.2)	332.1 (297.5 – 363.8)	396.5 (363.8 – 441.7)	510.4 (441.7 – 929.9)	
Univariate OR (95% CI)	9.34 (4.98 – 17.51)	4.22 (2.24 – 7.96)	3.02 (1.65 – 5.53)	1.00	<0.001
Multivariate OR[a] (95% CI)	7.03 (3.14 – 15.71)	4.16 (1.89 – 9.14)	3.17 (1.46 – 6.87)	1.00	<0.001
Lutein + Zeaxanthin					
Cases/Controls, n	56/166	40/182	52/171	32/190	
Median (Range), µg/L	98.0 (25.8 – 119.2)	135.5 (119.3 – 153.1)	174.5 (153.2 – 196.2)	243.2 (196.7 – 529.9)	
Univariate OR (95% CI)	2.03 (1.25 – 3.29)	1.33 (0.80 – 2.23)	1.84 (1.13 – 3.01)	1.00	0.01
Multivariate OR[a] (95% CI)	2.10 (1.17 – 3.77)	1.09 (0.59 – 2.03)	2.16 (1.18 – 3.95)	1.00	0.06
β–cryptoxanthin					
Cases/Controls, n	61/161	48/174	37/186	34/188	
Median (Range), µg/L	60.8 (3.9 – 81.2)	103.9 (81.6 – 132.6)	171.8 (132.6 – 228.2)	339.7 (228.5 – 1778.4)	
Univariate OR (95% CI)	2.17 (1.33 – 3.53)	1.57 (0.94 – 2.62)	1.14 (0.68 – 1.91)	1.00	0.002
Multivariate OR[a] (95% CI)	2.61 (1.41 – 4.86)	2.00 (1.08 – 3.67)	1.40 (0.74 – 2.67)	1.00	0.002
Total lycopene					
Median (Range), µg/L	55.8 (5.4 – 75.0)	89.4 (75.0 – 108.7)	133.0 (108.8 – 160.7)	209.4 (162.0 – 724.5)	
Cases/Controls, n	56/166	47/175	39/184	38/184	
Univariate OR (95% CI)	1.64 (1.04 – 2.60)	1.30 (0.81 – 2.09)	1.03 (0.63 – 1.68)	1.00	0.03
Multivariate OR[a] (95% CI)	1.29 (0.75 – 2.22)	1.12 (0.65 – 1.94)	0.89 (0.50 – 1.57)	1.00	0.34

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α–carotene					
Median (Range), µg/L	39.1 (4.2 – 51.7)	62.3 (51.7 – 73.3)	89.1 (73.3 – 107.7)	144.6 (108.1 – 1282.8)	
Cases/Controls, n	53/169	53/169	38/185	36/186	
Univariate OR (95% CI)	1.65 (1.02 – 2.65)	1.64 (1.01 – 2.64)	1.06 (0.64 – 1.76)	1.00	0.02
Multivariate OR[a] (95% CI)	1.53 (0.86 – 2.75)	1.73 (0.97 – 3.03)	1.04 (0.56 – 1.93)	1.00	0.06
Total–β–carotene					
Median (Range), µg/L	56.4 (4.8 – 84.7)	108.2 (84.8 – 136.4)	162.1 (136.5 – 212.1)	305.9 (212.4 – 1452.1)	
Cases/Controls, n	54/168	42/180	44/179	40/182	
Univariate OR (95% CI)	1.51 (0.94 – 2.44)	1.07 (0.66 – 1.75)	1.15 (0.71 – 1.85)	1.00	0.16
Multivariate OR[a] (95% CI)	1.52 (0.86 – 2.71)	1.33 (0.75 – 2.34)	1.10 (0.62 – 1.97)	1.00	0.14

211   [a]Adjusted for matching factors (age and sex), BMI categories, socioeconomic status, heavy

212   alcohol consumption, tobacco use, isoniazid preventive therapy, TB history, comorbid disease,

213   self-reported DM, and index patient smear status.

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215   Tables 4 provides univariate and adjusted odds ratios for vitamin A deficiency. HHCs

216   with baseline vitamin A deficiency were at 10.42-fold increased risk of incident TB disease

217   (95% CI 4.01–27.05; P < 0.001). A statistical test for interaction between vitamins A and D

218   deficiencies was not significant (P for interaction = 0.28).

219   **Table 4. Vitamins A deficiency with Selected Risk Factors and Risk of TB Disease.**

	Univariate OR (95% CI) N = 889	P value	Multivariate OR[a] (95% CI) N = 822	P value
BMI Categories[b]				
Underweight	4.14 (1.32 – 12.94)	0.01	2.70 (0.35 – 20.99)	0.34
Overweight	0.39 (0.26 – 0.60)	<0.001	0.36 (0.22 – 0.60)	<0.001
Normal	1.00		1.00	
Socioeconomic Status[c]				
Low	1.69 (1.16 – 2.47)	0.01	1.69 (1.06 – 2.70)	0.03
Middle	1.00		1.00	
High	0.95 (0.59 – 1.55)	0.85	1.17 (0.66 – 2.09)	0.60
Vitamin A deficiency (< 200 µg/L)	7.65 (3.72 – 15.71)	<0.001	10.42 (4.01 – 27.05)	<0.001

220   [a]Adjusted for matching factors (age and sex), vitamin D levels, BMI categories, socioeconomic

221   status, heavy alcohol consumption, tobacco use, isoniazid preventive therapy, TB history,

222   comorbid disease, self-reported DM, and index patient smear status.

223   [b]N = 885

224   [c]N = 869

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226   Stratification by age showed that VAD increased the risk of TB disease in 10–19 year

227   olds nearly 20-fold (aOR 18.58; 95% CI 3.54–97.60; P = 0.001) and in ≥ 20 year olds by 10-fold

228   (aOR 10.21; 95% CI 2.43–42.91; P = 0.002) [Table 5]. Adolescents aged 10–19 years in the

229   lowest quartile of carotenoid levels were at higher risk for TB disease but there was no effect in

230   older HHCs.

231   **Table 5. Micronutrient Levels and Risk of TB disease Stratified by Age Category.**

	Age 10 – 19 Years		Age ≥ 20 years	
	Multivariate OR[a] (95% CI) N = 237	P value	Multivariate OR[a] (95% CI) N = 565	P value
Vitamin A < 200 µg/L	18.58 (3.54 – 97.60)	0.001	10.21 (2.43 – 42.91)	0.002
Vitamin A (µg/L)				
Q1	7.72 (2.06 – 28.91)	0.002	4.43 (1.83 – 10.73)	0.001
Q2	4.83 (1.23 – 18.92)	0.02	3.50 (1.46 – 8.40)	0.005
Q3	1.00		1.65 (0.70 – 3.88)	0.25
Q4	NA[b]		1.00	
		p value for trend = 0.003		p value for trend <0.001
Lutein + Zeaxanthin (µg/L)				
Q1	1.68 (0.59 – 4.76)	0.33	1.61 (0.78 – 3.31)	0.19
Q2	1.09 (0.37 – 3.20)	0.88	1.41 (0.67 – 2.96)	0.37
Q3	0.33 (0.08 – 1.33)	0.12	2.05 (1.01 – 4.17)	0.05
Q4	1.00		1.00	
		p value for trend = 0.12		p value for trend = 0.24
β–cryptoxanthin (µg/L)				
Q1	4.39 (1.33 – 14.46)	0.01	2.00 (0.95 – 4.25)	0.07
Q2	1.57 (0.46 – 5.34)	0.47	2.02 (1.00 – 4.08)	0.05
Q3	0.97 (0.27 – 3.44)	0.96	1.64 (0.77 – 3.50)	0.20
Q4	1.00		1.00	
		p value for trend = 0.02		p value for trend = 0.03
Total lycopene (µg/L)				
Q1	3.56 (1.16 – 10.92)	0.03	0.95 (0.50 – 1.82)	0.88
Q2	1.99 (0.65 – 6.13)	0.23	0.90 (0.46 – 1.76)	0.76
Q3	2.41 (0.71 – 8.20)	0.16	0.66 (0.33 – 1.32)	0.24

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Q4	1.00		1.00	
		p value for trend = 0.04		p value for trend = 0.88
α–carotene (µg/L)				
Q1	4.54 (1.26 – 16.32)	0.02	1.00 (0.49 – 2.03)	1.00
Q2	1.83 (0.53 – 6.30)	0.34	1.60 (0.81 – 3.15)	0.17
Q3	1.93 (0.55 – 6.75)	0.31	0.80 (0.38 – 1.68)	0.55
Q4	1.00		1.00	
		p value for trend = 0.03		p value for trend = 0.55
Total–β–carotene (µg/L)				
Q1	2.58 (0.82 – 8.07)	0.10	1.18 (0.58 – 2.39)	0.66
Q2	2.51 (0.76 – 8.32)	0.13	0.97 (0.48 – 1.95)	0.92
Q3	0.98 (0.27 – 3.48)	0.97	1.14 (0.59 – 2.24)	0.69
Q4	1.00		1.00	
		p value for trend = 0.05		p value for trend = 0.77

232   [a]Adjusted for matching factors (age and sex), BMI categories, socioeconomic status, heavy

233   alcohol consumption, tobacco use, isoniazid preventive therapy, TB history, comorbid disease,

234   self-reported DM, and index patient smear status.

235   [b]Categorized into tertiles because there were five or fewer cases in the highest quartile.

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237   To rule out the possibility that vitamin A deficiency in cases might have resulted from

238   metabolic derangements due to early active TB disease that was not clinically apparent, we

239   stratified by baseline infection status and found vitamin A levels remained strongly associated

240   with TB disease in both groups (Table 6). In a second sensitivity analysis in which we excluded

241   cases (and their matched controls) diagnosed within 90 days of index patient enrollment,

242   multivariable analysis also continued to show a strong inverse association between quartiles of

243   vitamin A and TB disease although the association with VAD was no longer statistically

244   significant (Table 6).

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247 **Table 6. Vitamin A Levels and Risk of TB disease Stratified by Baseline Infection Status**
248 **and for TB Diagnosed at least 90 days After Index Case Enrollment.**

	Cases/Controls	Multivariate OR[a] (95% CI)	P value
Negative TB Infection at baseline			
Vitamin A (µg/L)			
Q1	12/106	4.38 (1.24 – 15.47)	0.02
Q2	13/144	3.71 (1.12 – 12.33)	0.03
Q3	6/152	1.00	
Q4	NA[b]		
			p value for trend = 0.02
Vitamin A Deficient (<200 µg/L)	3/8	20.32 (2.91 – 141.70)	0.002
Positive TB Infection at baseline			
Vitamin A (µg/L)			
Q1	80/90	2.56 (1.17 – 5.58)	0.02
Q2	40/89	1.83 (0.85 – 3.92)	0.12
Q3	25/102	1.00	
Q4	NA		
			p value for trend = 0.02
Vitamin A Deficient (<200 µg/L)	21/8	5.31 (1.63 – 17.25)	0.01
TB diagnosed ≥ 90 days			
Vitamin A (µg/L)			
Q1	27/70	4.21 (1.20 – 14.74)	0.02
Q2	24/73	5.25 (1.44 – 19.10)	0.01
Q3	19/79	2.91 (0.87 – 9.77)	0.08
Q4	8/89	1.00	
			p value for trend = 0.02
Vitamin A Deficient (<200 µg/L)	3/8	2.15 (0.33 – 13.80)	0.42

249 [a]Adjusted for matching factors (age and sex), BMI categories, socioeconomic status, heavy

250 alcohol consumption, tobacco use, isoniazid preventive therapy, TB history, comorbid disease,

251 self-reported DM, and index patient smear status.

252 [b]Categorized into tertiles because there were five or fewer cases in the highest quartile.

253

254 Restricting the analysis to microbiologically confirmed cases further strengthened the

255 association between vitamin A levels and TB disease (Table 7). We were unable to stratify by

256 IPT use because few HHCs had received IPT.

257 **Table 7. Vitamin A levels and Risk of Microbiologically Confirmed TB Disease.**

	All participants		Age 10 – 19 Years		Age ≥ 20 years	
	Multivariate OR[a] (95% CI) N = 666	P value	Multivariate OR[a] (95% CI) N = 178	P value	Multivariate OR[a] (95% CI) N = 487	P value
Vitamin A (µg/L)						
Q1	7.51 (2.75 – 20.54)	<0.001	8.30 (1.82 – 37.86)	0.01	6.95 (2.44 – 19.79)	<.001
Q2	5.71 (2.22 – 14.67)	<0.001	2.56 (0.49 – 13.52)	0.27	4.97 (1.89 – 13.07)	.001
Q3	3.58 (1.40 – 9.19)	0.01	1.00		1.60 (0.59 – 4.29)	.35
Q4	1.00		NA[b]		1.00	
		p value for trend <0.001		p value for trend = 0.002		p value for trend <0.001
Vitamin A Deficient (<200 µg/L)	16.65 (5.36 – 51.77)	<0.001	21.41 (3.71 – 123.48)	0.001	16.23 (3.12 – 84.44)	<0.001

258 [a]Adjusted for matching factors (age and sex), BMI categories, socioeconomic status, heavy

259 alcohol consumption, tobacco use, isoniazid preventive therapy, TB history, comorbid disease,

260 self-reported DM, and index patient smear status.

261 [b]Categorized into tertiles because there were five or fewer cases in the highest quartile.

262

263 **Discussion**

264 Here, we found that vitamin A levels strongly predict progression to TB disease in a

265 cohort of Peruvians with household exposure to an index TB patient. This relationship remained

266 strong after adjustment for socioeconomic status, BMI and other comorbidities that might be

267 associated with increased TB disease risk and lower vitamin A levels. Although the association

268 is most extreme at levels consistent with a diagnosis of VAD, we observed a dose-response

269 relationship with significantly increased risk of TB disease even at levels usually classified as

270 normal. Carotenoids were also inversely associated with risk of TB progression among

271 adolescents, although the effect of individual carotenoids was less extreme than for vitamin A.

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272   Although previous studies have documented low vitamin A levels among patients with

273   TB disease [8,9,29,30], the causal direction of this association has been unclear. TB disease can

274   lead to profound weight loss, and micronutrient deficiencies observed in patients often resolve

275   with TB treatment [30]. To date, no published studies have reported on the impact of vitamin A

276   supplementation on incident TB disease, and no clinical trials have demonstrated an impact of

277   supplementation on TB treatment outcomes [30].

278   In contrast to the plethora of work on micronutrients and TB treatment outcomes [30],

279   few studies have prospectively investigated the role of pre-existing vitamin A status in the

280   development of TB disease. Cegielski et al. observed that less than 2% of the US-based

281   NHANES 1 cohort [14] had baseline vitamin A levels under 300 µg/L (1.05 µmol/L), and only

282   61 people developed TB disease over 20 years of follow-up. Although TB incidence was nearly

283   three-fold higher among those with low vitamin A levels, confidence intervals for the hazard

284   ratio spanned from 0.70 to 11.4. These results are consistent with a study conducted in

285   Philadelphia between 1942–49, in which African American men with low baseline vitamin A

286   levels [15] were three times more likely to develop TB disease than those with higher levels.

287   Interestingly, the mean vitamin A level was 300 µg/L (1.05 µmol/L), suggesting that the

288   comparison was between men with low and very low levels, rather than among people with the

289   spectrum of levels we observed in Lima. This study also found a very strong effect of vitamin C

290   deficiency, with all those who developed TB disease having baseline vitamin C levels below 6

291   mg/L (34.07 µmol/L).

292   Multiple other lines of evidence suggest an association between vitamin A levels and TB

293   risk. In vitro studies have demonstrated that vitamin A and its metabolites cause dose-dependent

294   inhibition of *Mycobacterium tuberculosis* (Mtb) growth in culture [31,32]. Experimental studies

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295   in animal and cell models of TB infection also provide support for the protective role of retinol.

296   Although early studies in various models gave conflicting results [33–35], more recent studies in

297   mice [36], rats [37], THP-1 cells [38], and cultured human macrophages [39] show reduced Mtb

298   bacillary loads after treatment with retinoic acid. Interestingly, the dose of retinoic acid required

299   to produce a protective effect in human macrophages is lower when it was added prior to

300   infection than when it was added afterwards [39].

301   Vitamin A also affects the immune system in various ways relevant to the pathogenesis

302   of TB [40]. Most of its effects are exerted by retinoic acid, which binds to the nuclear receptors

303   RAR and RXR to control transcriptional expression of numerous target genes. Among its

304   recently recognized effects on immunity, retinol modulates the maturation, antigen presentation,

305   migration, and T-cell priming functions of dendritic cells [41–43], as well as the generation of T-

306   regulatory cells and suppression of Th17 cells [44]. Retinoic acid has also been shown to be

307   crucial to the maintenance of the Th1 lineage [45], an essential component of an effective

308   immune response to tuberculosis.

309   In addition to its impact on acquired immunity, recent evidence suggests that vitamin A

310   may play a role in innate immune responses to Mtb. Wheelwright et al. found that all-trans-

311   retinoic acid exerts an antimicrobial effect on Mtb in human monocytes through its effect on

312   cellular cholesterol efflux mediated through the NPC2 gene [6]. Stimulation of primary human

313   monocytes with all trans-retinoic acid increased expression of NPC2 and reduced cellular

314   cholesterol through a mechanism that is distinct from the antimicrobial activity of 1,25-

315   dihydroxyvitamin D3 [6]. Mtb has been shown to use host lipids as a nutrient source, and host

316   cholesterol mediates Mtb persistence and survival within macrophages [46]. Taken together,

317   these findings suggest that low vitamin A levels may impair host's ability to control TB infection

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318   after exposure.

319   Our study had notable limitations. Participants were followed for one year, a relatively

320   short period of time given the slow pathogenesis of tuberculosis. Because we had relatively few

321   blood samples from children under ten, we were unable to assess the impact of vitamin A

322   deficiency in that age group. Almost all our study participants had been vaccinated with BCG so

323   we are unable to distinguish between the possibilities that vitamin A enhanced BCG-induced

324   adaptive immunity or provided direct protection against TB progression. Although we found no

325   evidence of an interaction between vitamin A and vitamin D on TB disease risk, our study may

326   not have been powered to detect such an interaction.

327   Most importantly, despite our efforts to ensure that nutritional assessments occurred prior

328   to the development of TB disease, we could not rule out the possibility that participants had

329   unrecognized TB disease at baseline, which may have led to the low retinol levels we observed.

330   We addressed this issue by conducting two sensitivity analyses, one of which stratified on

331   baseline infection status and one which excluded cases diagnosed within 90 days of index patient

332   enrollment. In both analyses, risk of TB disease remained inversely associated with vitamin A

333   quartiles regardless of baseline TB infection status or timing of diagnosis. Nevertheless, since

334   active TB may remain clinically undetected for months, especially in children, and since acute

335   infections are known to affect serum vitamin A levels, there is no way to rule out reverse

336   causality without conducting a clinical trial of vitamin A supplementation in people at high risk

337   for TB.

338   In conclusion, we find that vitamin A levels among persons exposed at home to a TB

339   patient strongly predicted incident TB disease within 12 months of follow-up in a dose-

340   dependent manner. Our data raise the possibility that screening for vitamin A levels among

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341   people at high risk for TB could help identify those likely to develop disease in the near future so

342   they could be targeted for early intervention. In the event that the association between VAD and

343   tuberculosis progression proves to be causal, routine vitamin A supplementation among persons

344   at high risk for developing TB disease (e.g. household contacts of TB cases or individuals in

345   congregate settings such as prisons) may provide an inexpensive, safe and effective means of

346   preventing progression from TB infection to TB disease.

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