Implications of reference equations on interpretation of spirometry among members of TB-affected households in three African countries

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Supplementary methods

Spirometry procedures

Spirometry was performed according to a standard operating procedure developed according to European Respiratory Society/American Thoracic Society (ERS/ATS) equations by Pan-African Thoracic Society (PATS)-trained nurses.(1) It was performed at the baseline study visit unless contraindicated (e.g. presence of tuberculosis (TB)-related symptoms, participant declined). In addition, for periods in

2021, we suspended spirometry due to infection prevention and control considerations, in the context of high local prevalence of COVID-19. In these cases, spirometry was performed at the next study visit.

All spirometry traces were manually quality-assured in real time by the trained operator and underwent external quality assurance in duplicate (EM and LZ). Each of FEV1 and FVC were separately assessed for useability and acceptability, and quality graded using repeatability and acceptability criteria (A-F, or U) as per 2019 ATS/ ERS grading system.(1)

Definition of healthy used in this study

All participants underwent screening for tuberculosis. This comprised a World Health Organization symptom screen(2) and a chest radiograph. If either, raised suspicion of TB, Xpert Mtb/Rif Ultra (Cepheid) was performed. If this was positive, further samples were taken for Xpert and culture. TB diagnoses could be either microbiologically confirmed (i.e. based on Xpert and/or culture results) or clinical (based on assessment by a clinical officer, in the absence of positive microbiology) – all were excluded from this analysis

Height was measured using a fixed stadiometer and weight using calibrated scales (Seca).

Participants were defined a ‘healthy’ for the purpose of evaluation of spirometry reference if they met all the following criteria: non-smoker, or former smoker with <1 cumulative pack year smoking history; no self-reported lung disease (including ever having had pneumonia or TB); not diagnosed with co-prevalent TB or incident TB during follow up; BMI in the normal range.

Normal BMI was defined for adolescents (<19 years) as BMI-for-age Z-score of between -2 and +2, calculated using sex-specific World Health Organization (WHO) reference equations, and for adults as BMI $\geq$18.5kg/m^2 and <25.0kg/m^2.

Ethical approvals

Ethical approval for the study was granted by the Medical Research Council in Zimbabwe (MRCZ/A/2618), the Mbeya Medical Research and Ethics Review Committee (SZEC-2439/R.A/V.1/101), the National Health Research Ethics Committee in Tanzania NIMR/HQ/R.8a/Vol.IX/3608) and Tanzanian Medicines and Medical Devices Authority (TMDA-WEB0021/CTR/0004/03), the National Bioethics Committee for Health in Mozambique (541/CNBS/21), and the ethical committees of London School of Hygiene & Tropical Medicine, United Kingdom (22522–2) and the medical faculty of the Ludwig Maximilian-Universität München, Germany (20–0771).

Comparison to other African reference equations

We sought to explore whether our data fit better to reference equations developed in Africa, compared to those from GLI. Relevant equations were identified by scoping review. We searched Medline on 28th January 2024, with no data restrictions, using terms for spirometry/lung function, healthy, and an expert-developed search for all countries in Africa. We considered articles where the primary objective was to develop a reference equation for spirometry interpretation and both male and female participants from a wide age range (i.e. not restricting to either older adults or children) were included. We did not consider articles published prior to 1990, given the recent advancements in equipment and standardization of procedures. From 72 returned results, we identified four studies (from Cameroon, Mozambique, Madagascar and Nigeria).(3–6) Three only recruited adults (age ≥18) with varying upper age limits, one included both children and adults (Cameroon). Our searches additionally identified a paper evaluating children in Madagascar, however this only recruited children across a narrow age range (8-12 years) and therefore was not considered relevant to our adolescent population.(7)

Three of the four papers used a linear model as the basis of prediction equations, concluding that more complex models did not confer better fit compared to this approach in their data. We used the authors reported equations to calculate Z scores for our study population.

Table E1: Characteristics of studies describing development of African reference equations for interpretation of spirometry

Country (reference)	N participants	Age range (years)	Type of model
Cameroon(5)	1777	4-88	GAMLSS
Madagascar(6)	2491	18-73	Linear
Mozambique(3)	155	18-NS	Linear
Nigeria(4)	720	18-65	Linear

Footnotes: GAMLSS = Generalized Additive Models for Location Scale and Shape; NS = not stated

Supplementary results

Table E2a: Characteristics of participants with and without a valid spirometry result

Characteristic	No/low quality spirometry N = 577*	Spirometry N = 1,532	p-value
Age, years	33 (19, 50)	25 (16, 39)	<0.001
Sex			0.005
Female	387 (67%)	925 (60%)	
Male	190 (33%)	607 (40%)	
HIV	88 (15%)	235 (15%)	>0.9

Footnotes: Presented as median (interquartile range) or number (percentage). P-values are from Wilcoxon rank sum tests (continuous variables) and chi-squared tests (binary variables). * Spirometry was not done for 293 participants and was of low quality for 175. **Abbreviations**: N = number.

Table E2b: Characteristics of participants categorized as healthy, compared to those categorized as not healthy

Characteristic	Healthy population N = 806	Not healthy population N = 726	p-value
Age, years	18 (14, 29)	34 (23, 46)	<0.001
Sex			0.001
Female	456 (57%)	469 (65%)	
Male	350 (43%)	257 (35%)	
HIV	79 (9.8%)	156 (22%)	<0.001

Footnotes: Presented as median (interquartile range) or number (percentage). P-values are from Wilcoxon rank sum tests (continuous variables) and chi-squared tests (binary variables). **Abbreviations**: N = number.

Table E3a: Characteristics of study population by site

Characteristic	Overall N = 806	Harare N = 247	Maputo N = 279	Mbeya N = 280	p-value
Age (years)	18 (14, 29)	19 (14, 28)	18 (15, 27)	18 (14, 30)	0.7
Age category					0.4
10-17 years	384 (48%)	112 (45%)	133 (48%)	139 (50%)	
18-39 years	333 (41%)	110 (45%)	119 (43%)	104 (37%)	
40+ years	89 (11%)	25 (10%)	27 (9.7%)	37 (13%)	
Sex					0.2
Female	456 (57%)	151 (61%)	151 (54%)	154 (55%)	
Male	350 (43%)	96 (39%)	128 (46%)	126 (45%)	
Highest educational level					<0.001
None or primary school	336 (43%)	72 (30%)	88 (33%)	176 (65%)	
At least secondary school	439 (57%)	168 (70%)	175 (67%)	96 (35%)	
Insufficient food*	189 (23%)	75 (30%)	102 (37%)	12 (4.3%)	<0.001
HIV†	79 (9.8%)	20 (8.2%)	29 (10%)	30 (11%)	0.6
On ART	62 (78%)	16 (80%)	25 (86%)	21 (70%)	0.3
Diabetes‡	29 (7.2%)	6 (4.8%)	9 (6.3%)	14 (10%)	0.2
Hypertension‡	76 (19%)	26 (20%)	26 (18%)	24 (19%)	>0.9
Height-for-age Z-score§	-0.76 (-1.40, -0.02)	-0.55 (-1.15, 0.13)	-0.36 (-1.09, 0.28)	-1.21 (-1.86, -0.63)	<0.001
BMI-for-age Z-score§	-0.18 (-0.82, 0.41)	-0.04 (-0.64, 0.59)	-0.36 (-0.91, 0.33)	-0.13 (-0.87, 0.36)	<0.001

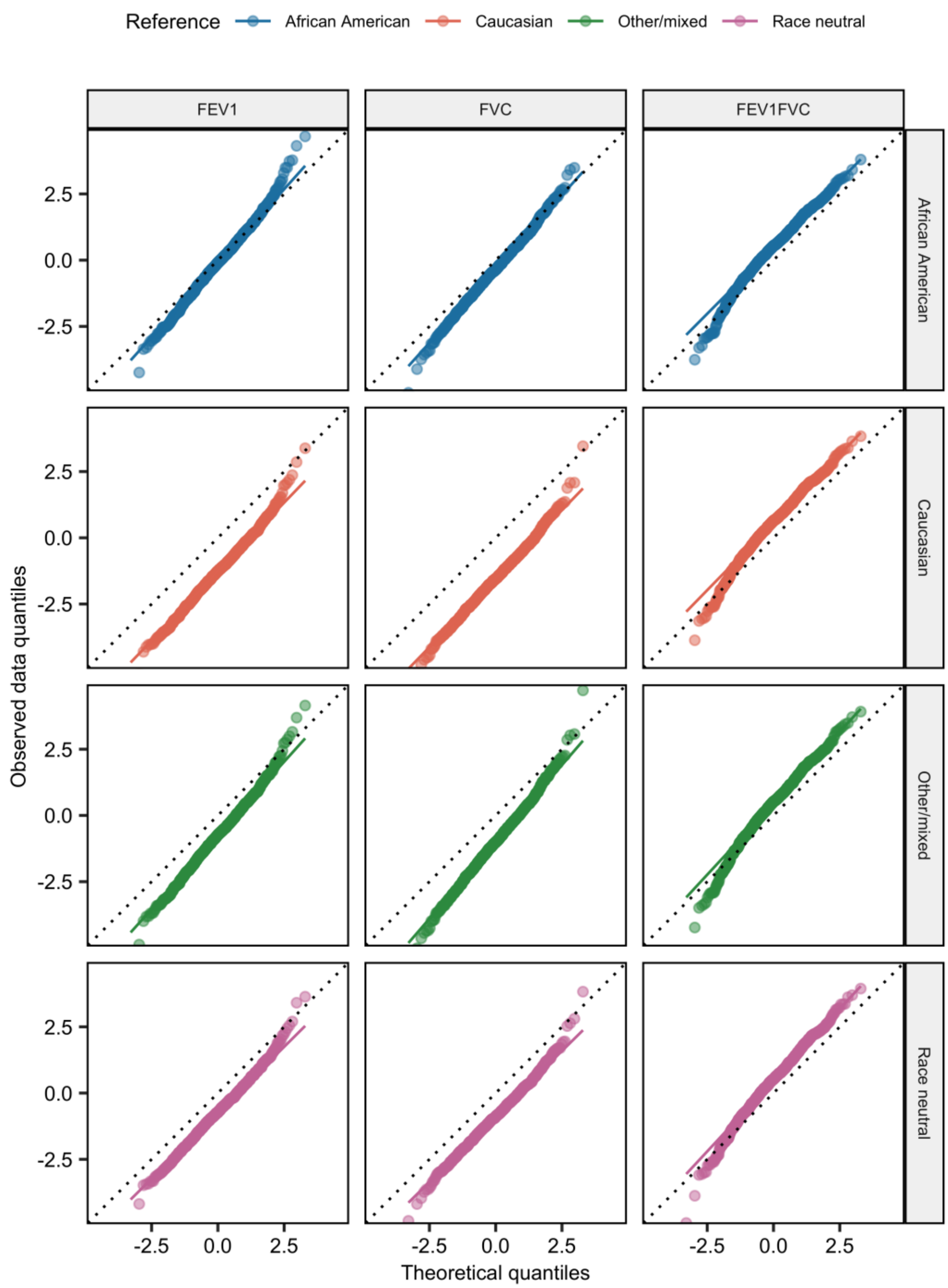
Footnotes: Presented as median (interquartile range) or number (percentage). P-values are from Wilcoxon rank sum tests (continuous variables) and chi-squared tests (binary variables). * Insufficient food from participant self-report (was there any day in the past six months where you did not have enough food. † 3 people were missing HIV status, denominator for anti-retroviral therapy (ART) is the number of people with HIV. ‡ Diabetes and hypertension status are based on either a self-reported diagnosis, report of being on medication for these conditions, or an elevated HbA1c or blood pressure, respectively. § Body mass index (BMI) and height for age Z-scores were calculated using the World Health Organization reference population, with the highest category (19 years) used as the reference value for all adults. **Abbreviations**: N = number.

Table E3b: Characteristics of study households by site (N=550)

Characteristic	Overall N = 466	Harare N = 141	Maputo N = 162	Mbeya N = 163	p-value
Stove					<0.001
Clean stove	402 (86%)	141 (100%)	148 (91%)	113 (69%)	
Solid fuel stove	64 (14%)	0 (0%)	14 (8.6%)	50 (31%)	
Household income per person per day <1.90 USD*	357 (92%)	115 (86%)	132 (92%)	110 (97%)	0.009

Footnotes: presented as number (percentage). * 1.90 United States Dollars (USD) was the international poverty line at the time when recruitment to the study started. Income was not known for 78 households. **Abbreviations**: N = number.

Figure E1: QQ plot of fit of Global Lung Initiative reference equations among healthy members of tuberculosis-affected households (N=806)



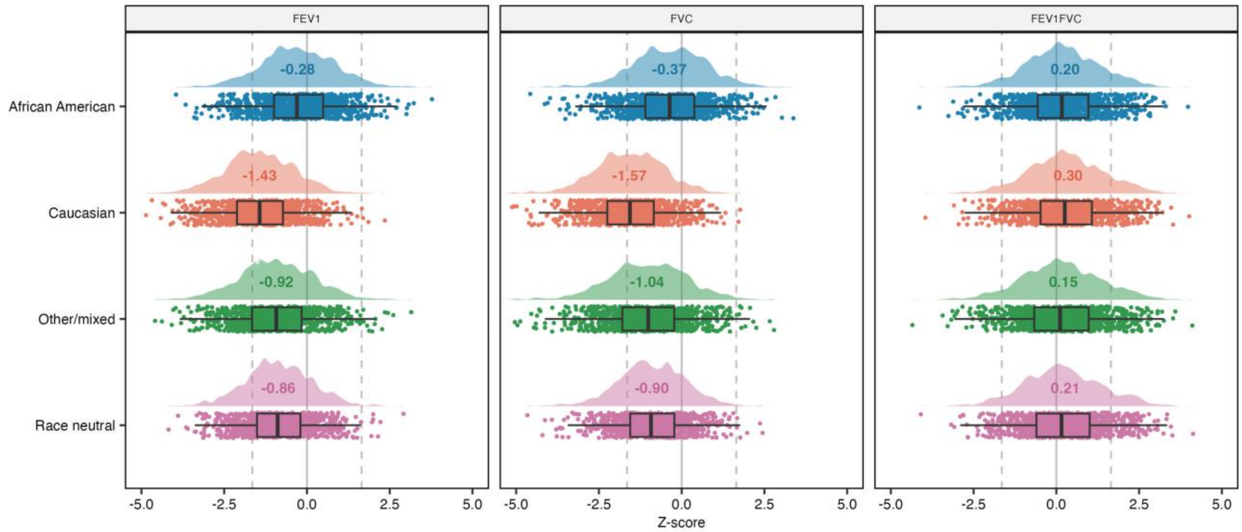
Abbreviations: FEV1 = Forced Expiratory Volume in one second, FVC = Forced Vital Capacity.

Table E4: Mean and standard deviation of key spirometric indices by different reference equations (N=806)

	Reference equation	FEV1	FVC	FEV1/FVC
GLI	African American	-0.12 (1.19)	-0.35 (1.15)	0.39 (1.16)
	Caucasian	-1.30 (1.11)	-1.57 (1.09)	0.59 (1.14)
	Other/mixed	-0.78 (1.19)	-1.03 (1.23)	0.46 (1.20)
	GLI Global	-0.73 (1.07)	-0.90 (1.04)	0.52 (1.16)
African*	Cameroon	0.07 (1.12)	0.01 (1.01)	0.17 (1.32)
	Madagascar	-0.19 (1.37)	-0.32 (1.36)	0.21 (1.25)
	Mozambique	0.26 (1.28)	-0.10 (1.30)	0.77 (1.07)
	Nigeria	0.06 (0.81)	0.05 (1.04)	0.18 (0.76)

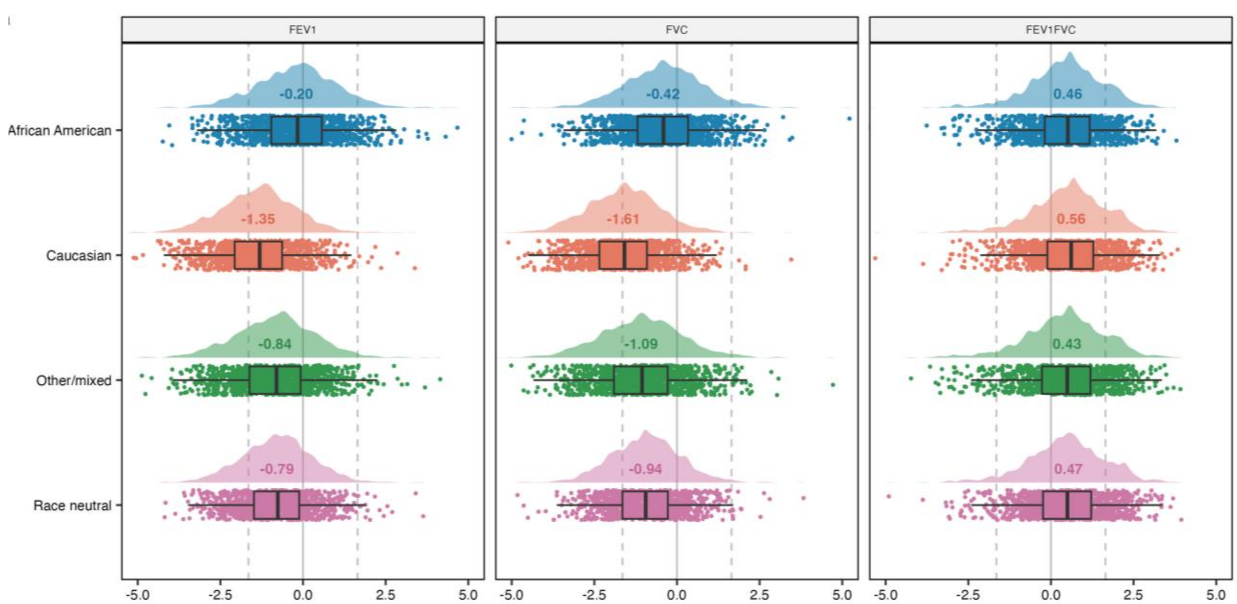
Footnotes: presented as mean (standard deviation). African equations were identified through scoping review. **Abbreviations:** FEV1 = Forced Expiratory Volume in one second, FVC = Forced Vital Capacity.

Figure E2a: Fit of GLI reference equations among healthy members of tuberculosis-affected households, using pre-bronchodilator spirometry (N = 806)



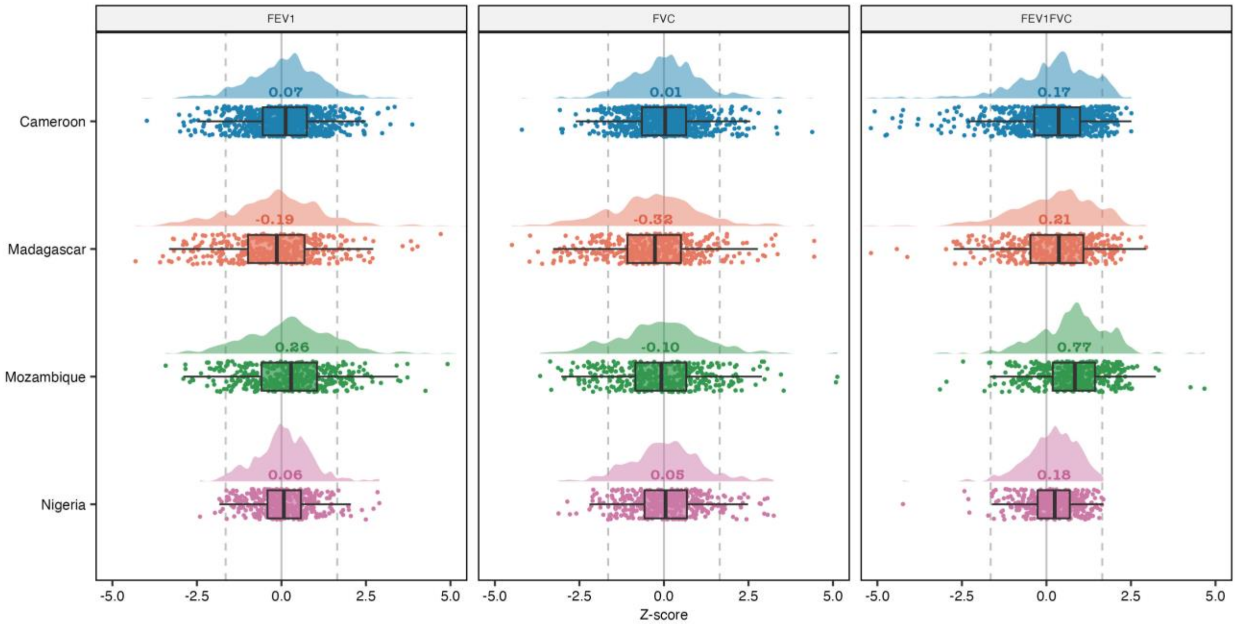
Footnotes: Fit of various Global Lung Function Initiative (GLI) reference equations to the healthy population in ERASE-TB across key indices (forced expiratory volume in 1 second [FEV1], forced vital capacity [FVC] and ratio of FEV1 to FVC). Annotations above box plots indicate mean Z-score.

Figure E2a: Fit of GLI reference equations among members of tuberculosis-affected households, not restricted to those with BMI in the normal range (N = 1214)



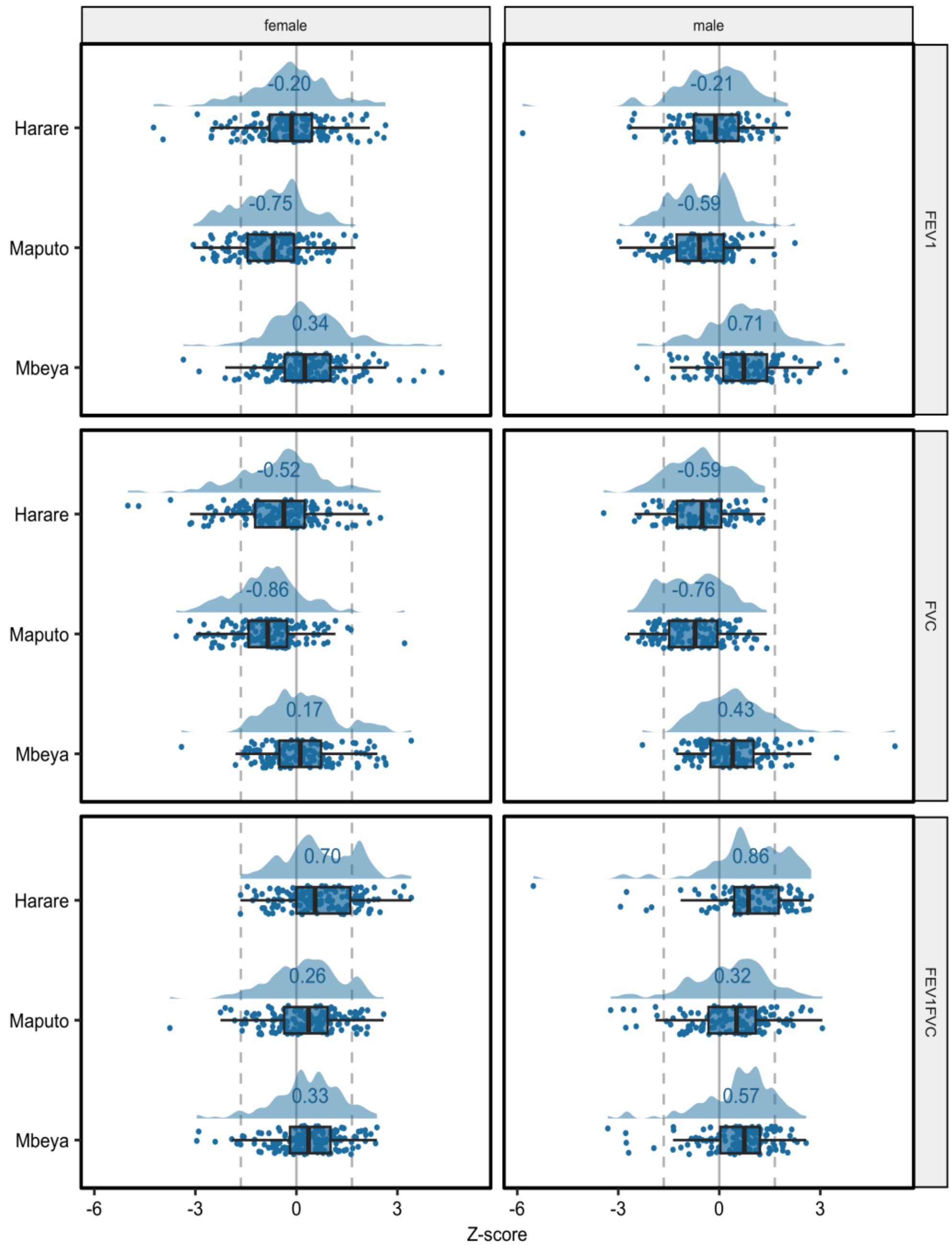
Footnotes: Fit of various Global Lung Function Initiative (GLI) reference equations to the ERASE-TB population across key indices (forced expiratory volume in 1 second [FEV1], forced vital capacity [FVC] and ratio of FEV1 to FVC). Annotations above box plots indicate mean Z-score. In contrast to primary analyses, here participants with body mass index (BMI) outside the normal range (i.e. adolescents with BMI < -2 or > +2 and adults with BMI $<18.5 kg/m^2$ or $\geq 25 kg/m^2$ were included.

Figure E3: Distribution of post-bronchodilator Z-scores among healthy members of tuberculosis-affected households, using African reference equations identified from scoping review (N=806)



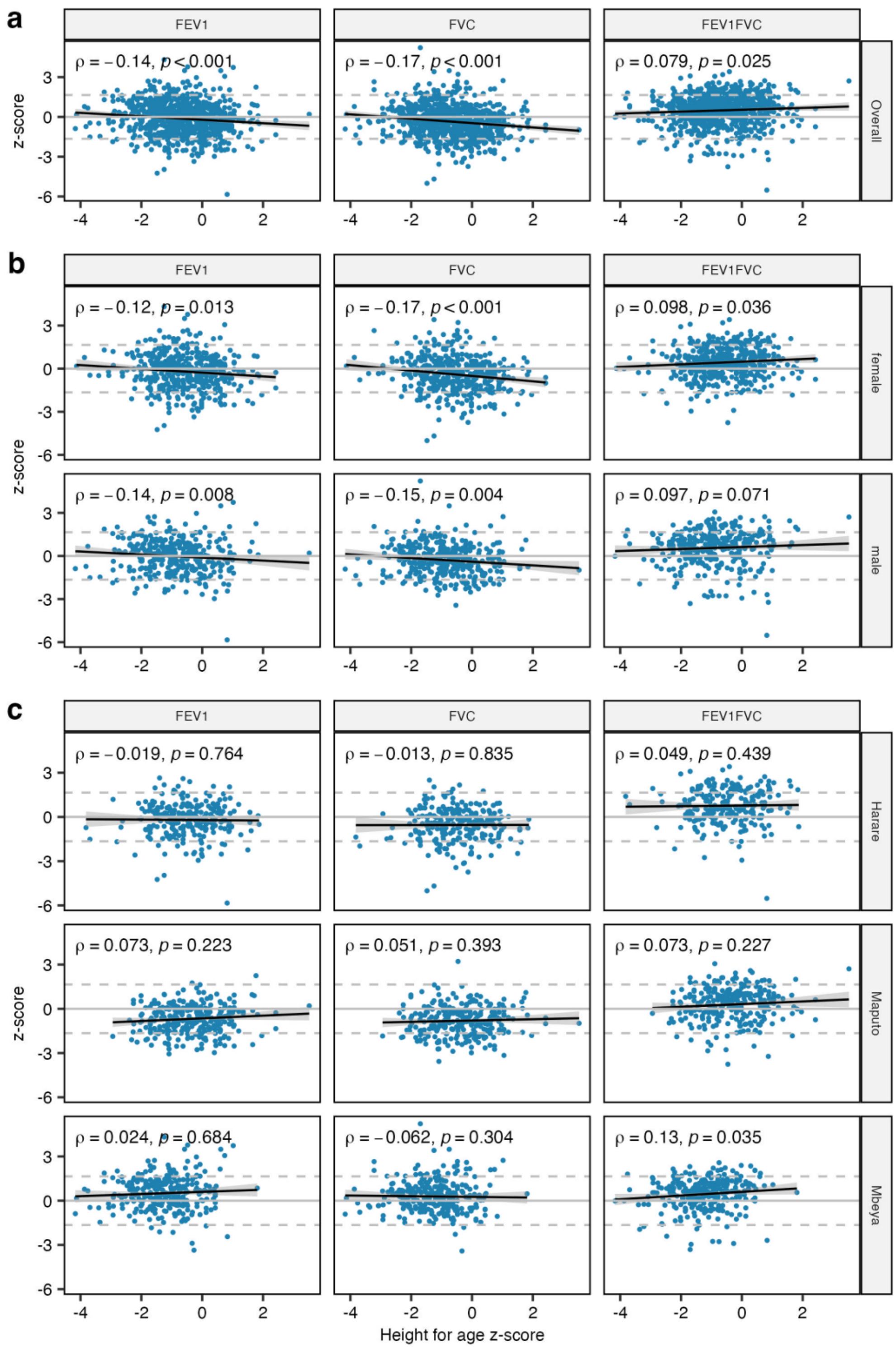
Footnotes: Fit of African reference equations; restricted to healthy adults (N=631) to reflect the population in which reference equations were derived. Annotations above box plots indicate mean Z-score.

Figure E4: Distribution of FEV1, FVC and FEV1/FVC Z-scores stratified by site and sex.



Footnotes: Z scores calculated using African American reference equation. Annotations above box plots indicate mean Z-score. **Abbreviations:** FEV1 = Forced Expiratory Volume in one second, FVC = Forced Vital Capacity.

Figure E5: Association between FEV1, FVC and FEV1/FVC Z-scores and height for age Z-score, overall (a) and stratified by sex (b) and study site (c).



Footnotes: Correlation coefficients and p values calculated using Spearman rank correlation. Height for age Z scores were calculated using WHO reference values for children and adolescents 5-19 years. The values for 19 year olds were used to calculate Z-scores for all adults. **Abbreviations:** FEV1 = Forced Expiratory Volume in one second, FVC = Forced Vital Capacity.

Table E5: Association between biomass exposure and lung function parameters among participants in Tanzania (N=280)

	Mean (SD) Z score		Coefficient	
Measure	**Clean**	**Biomass**	**Unadjusted**	**Adjusted***
FEV1	0.56 (1.08)	0.50 (1.12)	-0.49 (-0.77, -0.20)	-0.49 (-0.78, -0.19)
FVC	0.24 (1.19)	0.29 (1.03)	-0.28 (-0.55, -0.01)	-0.23 (-0.51, 0.06)
FEV1/FVC	0.46 (1.10)	0.36 (1.03)	-0.40 (-0.66, -0.14)	-0.47 (-0.74, -0.20)

Footnotes: *** Coefficients are from linear regression models, adjusted estimates were adjusted for Age (10–17 years, 18–39 years, 40+ years), Sex, HIV status, SES quintile. Abbreviations**: FEV1 = Forced Expiratory Volume in one second, FVC = Forced Vital Capacity, SD = standard deviation.

References

1. Graham BL, Steenbruggen I, Barjaktarevic IZ, Cooper BG, Hall GL, Hallstrand TS, *et al*. Standardization of spirometry 2019 update an official American Thoracic Society and European Respiratory Society technical statement. *American Journal of Respiratory and Critical Care Medicine* 2019;200:E70–E88.
2. World Health Organization. *WHO consolidated guidelines on tuberculosis. Module 2: screening – systematic screening for tuberculosis disease*. Geneva: World Health Organization; 2021.
3. Ivanova O, Khosa C, Bakuli A, Bhatt N, Massango I, Jani I, *et al*. Lung Function Testing and Prediction Equations in Adult Population from Maputo, Mozambique. *International Journal of Environmental Research and Public Health* 2020;17:4535.
4. Fawibe AE, Odeigah LO, Saka MJ. Reference equations for spirometric indices from a sample of the general adult population in Nigeria. *BMC Pulmonary Medicine* 2017;17:48.
5. Pefura-Yone EW, Balkissou AD, Poka-Mayap V, Djenabou A, Massongo M, Ofimboudem NA, *et al*. Spirometric reference equations for Cameroonians aged 4 to 89 years derived using lambda, mu, sigma (LMS) method. *BMC Pulm Med* 2021;21:344.
6. Ratomaharo J, Perdomo OL, Collingridge DS, Andriamihaja R, Hegewald M, Jensen RL, *et al*. Spirometric reference values for Malagasy adults aged 18–73 years. *European Respiratory Journal* 2015;45:1046–1054.
7. Wolff PTh, Arison L, Rahajamiakatra A, Raserijaona F, Niggemann B. Spirometric reference values in urban children in madagascar: Poverty is a risk factor for low lung function. *Pediatric Pulmonology* 2014;49:76–83.