Evaluation of the Global Lung Initiative 2012 Reference Values for Spirometry in African Children

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Abstract

Rationale: Despite the high burden of respiratory disease, no spirometry reference values for African children are available.

Objectives: Investigate whether the Global Lung Initiative (GLI-2012) reference values for spirometry are appropriate for children in sub-Saharan Africa and assess the impact of malnutrition on lung function.

Methods: Anthropometry and spirometry were obtained in children aged 6 to 12 years from urban and semiurban schools in three African countries. Spirometry *z*-scores were derived using the GLI-2012 prediction equations for African Americans. Thinness (body mass index *z*-score < -2) was a surrogate for malnutrition. Spirometry outcomes were compared with those of African American children from the third National Health and Nutrition Survey.

Measurements and Main Results: Spirometry data were analyzed from 1,082 schoolchildren (51% boys) aged 6.0 to 12.8 years in Angola (n = 306), Democratic Republic of the Congo (n = 377), and Madagascar (n = 399). GLI-2012 provided a good fit with mean (SD) *z*-scores of −0.11 (0.83) for FEV_1, −0.08 (0.86) for FVC, and −0.07 (0.83) for FEV_1/FVC. Due to low scatter, the fifth centile corresponded to −1.3 *z*-scores in boys and −1.5 *z*-scores in girls. Malnourished African children had a normal FEV_1/FVC ratio but significant reductions of ∼0.5 *z*-scores (∼5%) in FEV_1 and FVC compared with African American peers from the third National Health and Nutrition Survey. Children in Angola had the lowest, and those in Madagascar had the highest, *z*FEV_1 and *z*FVC.

Conclusions: The results of this study support the use of GLI-2012 reference values for schoolchildren in sub-Saharan Africa. Malnutrition affects body growth, leading to a proportionately smaller FEV_1 and FVC without respiratory impairment, as shown by the normal FEV_1/FVC ratio.

Keywords: spirometry; pediatrics; reference values; Africa; malnutrition

Interpretation of pulmonary function test results requires considering age, sex, height, and ethnic group. In 2012, the Global Lung Function Initiative (GLI-2012) produced a prediction equation model for spirometry that fits four ethnic groups (1): white, African American, Southeast Asian, and Northeast Asian, plus a provisional "other" group representing other populations and individuals of mixed ethnic origin. That study confirmed that for the same age, height, and sex, people of European ancestry have larger lung volumes than African Americans (2–4), reflecting genetically and environmentally determined differences in body frame (black people have larger limbs relative to stature [5–7], which has a genetic basis [7, 8]). GLI-2012 does not fit a North African population with Eurasian and sub-Saharan ancestry (9). It is unknown whether the

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At a Glance Commentary

Scientific Knowledge on the Subject: There is a high burden of lung disease in sub-Saharan Africa, yet no spirometry reference equations validated across different countries are available for evaluating lung function of African schoolchildren. The effect of malnutrition on lung function in children is also poorly defined.

What This Study Adds to the Field: Despite large differences in socioeconomic development between the United States and sub-Saharan Africa, Global Lung Initiative 2012 reference values for spirometry for African Americans are appropriate for well-nourished children attending school in Angola, Democratic Republic of the Congo, and Madagascar. The FEV_1 and FVC were reduced in malnourished children, but the reduction was proportional, as indicated by a normal FEV_1/FVC ratio. Malnutrition affects growth and hence chest size, leading to smaller lungs, but the normal FEV_1/FVC ratio indicates that there is no evidence of functional impairment.

GLI-2012 reference values for African Americans are appropriate for sub-Saharan African people (1). A good fit might be expected because of the shared genetic background. Two studies in adults (10, 11) and one in children (12), respectively from Rwanda, Madagascar, and Nigeria, suggest that lung volumes in these populations are comparable to those in African Americans. There are relevant differences between African American and African children in terms of genetic mixing (13, 14), average level of affluence, healthcare access, and exposure to air pollutants. Many children living in sub-Saharan Africa suffer the consequences of poverty and impaired nutritional status. The impact of these factors on lung function in children is controversial. Some studies found a relationship between low socioeconomic status (SES), malnutrition, and lung volumes (15–22), but others did not (23–25). Moreover, after considering differences in body frame and genetic ancestry, disparities in SES explain only a small proportion of differences in lung function (6, 7, 17, 18, 23). This suggests that SES and nutrition act through an effect on body growth (26, 27). In sub-Saharan Africa, almost 90% of the people rely on biomass fuels, mainly wood, to meet their domestic energy demands (28). The solid fuel smoke causes high levels of indoor air pollution. Children in urban areas are also exposed to higher levels of outdoor air pollution than in American cities (29). These exposures increase the risk of serious acute respiratory infections in young children (28) and may permanently affect lung health and spirometry outcomes (30). Therefore, the "GLI-2012 black" prediction equations might not fit African children. Appropriate spirometry reference data for sub-Saharan African children are urgently needed, considering the high burden of childhood respiratory disease.

This study aimed to test the applicability of GLI-2012 prediction equations for African American children to data collected cross-sectionally from primary school children in Angola (South-West Africa), Democratic Republic of Congo (DR Congo, Central Africa), and Madagascar (southeast African island). Secondary aims were to evaluate the impact of malnutrition on lung function and compare respiratory outcomes in African children and African American peers from NHANES III (National Health and Nutrition Examination Survey III) (31). Some of the results of this study have been previously reported in abstract form (32, 33).

Methods

This prospective cross-sectional multicenter study was conducted in Angola, DR Congo, and Madagascar by the principal investigator (PI) and various coinvestigators in different countries, using identical equipment, techniques, and quality control criteria.

The study was approved by the Ethics Committee of the University Campus Bio-Medico of Rome, Italy and by the respective local committees of collaborating hospitals in the three African countries. Parental written consent and verbal assent from each child were obtained in their first language before assessments.

Subjects

Apparently healthy school children 6 to 12 years old were eligible. Data were collected between October 2012 and May 2015 in Ambanja (Madagascar), Luanda (Angola), and Kinshasa (DR Congo), in two public and two private schools in each country (details in online supplement). Angola and DR Congo are mainly populated by genetically quite homogenous ethnic groups of Bantu west-central African origin (34, 35). The Malagasy population shows a combination of morphological and cultural traits typical of Bantu and Austronesians (36, 37). We collected data in the northwestern coastal area of Madagascar, inhabited by Sakalava people, a negroid group with prevalent "African features" but mixed Bantu-Austronesian genetic background (37).

Logistic limitations precluded enrolling children not attending school. Children from Angola and DR Congo lived in urban areas with high levels of indoor and outdoor air pollution (29), and Malagasy children were from a semiurbanized area with less outdoor air pollution.

Children with respiratory symptoms (i.e., cough, coryza) or suspected fever on the test day were excluded. The PI evaluated the presence of symptoms and performed cardiac auscultation before the spirometric test. Also, children with current asthma or known chronic conditions likely to influence lung function or spirometry performance (e.g., congenital heart disease, mental retardation, previous tuberculosis) were excluded. Current asthma was defined as the occurrence of at least one episode of wheezing or whistling in the chest in the last 12 months (38). This information was obtained from the children and their teachers. A local investigator performed the interview in the local first language and explained the meaning of the questions. Teachers were also asked if they were aware of any relevant chronic disease in the pupils.

Assessments

Age was recorded with one decimal accuracy. Weight and standing and sitting height were measured using the same protocol and instruments (additional details are available in the online supplement). The Cormic index was calculated as the sitting height/height ratio.

z-Scores for BMI (zBMI) and height (zHeight) were derived as a function of age for boys and girls using the lambda-mu-sigma parameters available from the CDC and World Health Organization websites (39, 40). "Thinness," defined as zBMI less than −2, was considered as a marker of

malnutrition. Stunted growth was defined as zHeight less than −2. For the purpose of this study, the presence of thinness or public school attendance were considered as pointers to lower SES.

All studies were performed with a Pony FX spirometer (Cosmed, Rome, Italy), which meets American Thoracic Society/European Respiratory Society (ATS/ERS) requirements (41). The principal investigator performed all the spirometric tests in the classrooms. Children performed two up to seven forced expiratory maneuvers standing upright with nose clip *in situ*. Data were included if there were at least two forced expiratory maneuvers meeting the ATS/ERS acceptability and repeatability criteria (41) as modified for children by Kirkby and colleagues (42), with a normally shaped flow-volume curve. All spirometry data were subjected to independent quality control by two different investigators experienced in spirometry.

Statistical Analyses

We sampled more than 150 boys and 150 girls in each country, more than needed to validate spirometric reference equations for each country (43). Spirometric variables from these children and from black American peers (6–13 yr) from the NHANES III study were converted to *z*-scores according to the GLI-2012 equations for African Americans, using the GLI-2012 software (http://www.ers-education.org/guidelines/global-lung-function-initiative/tools.aspx). Reference equations were derived with the lambda-mu-sigma method using the GAMLSS package (version 4.3–6) (44) and the statistical software R (version 3.2.2; The R Project for Statistical Computing, www.r-project.org), adopting the Box–Cox–Cole–Green distribution (45) or normal distribution. Models tested included an age spline and log transformations of spirometric index, age, and height. The general form of the model was:

$$Y = a + b \times \text{height} + c \times \text{age} + d \times \text{variable} + \text{age-spline},$$

where Y was the spirometric index; variable could be Cormic index, zBMI, sitting height, or country in which data had been collected; and a, b, c, and d were regression coefficients. Models were developed for boys and girls with both untransformed and log-transformed Y, height, and age.

The Bayesian information criterion was used to select the most parsimonious model. Goodness of fit was checked by inspection of Q-Q plots and worm plots (i.e., detrended Q-Q plots, which highlight departure from normality). In some analyses, residuals represented *z*-scores greater than ±4; because the analyses are sensitive to outliers, these were removed. Penalized β-splines were used to obtain smoothly changing curves over the entire age range. Separate models were developed for boys and girls. Models were also developed for height as a function of age, country of residence, and zBMI. Group differences were assessed by *t* tests, analysis of variance, Tukey's honestly significant difference test, and the Mantel-Haenszel chi-squared test for stratified tables. In the case of multiple testing, we controlled for a false discovery rate (46); otherwise, a P value < 0.05 was adopted as representing a statistically significant difference.

Results

In total, 1,328 children were enrolled. After exclusions, results from 1,082 subjects (mean ± SD age of 9.3 ± 1.7 yr, 51.9% boys) from Angola (n = 306), DR Congo (n = 377), and Madagascar (n = 399) were analyzed (Figure 1). Sex and age distributions were grossly comparable in the different countries (Table 1). Overall, 60% of children attended private schools (Table 1; *see* Table E1 in the online supplement).

Anthropometric Results

Children in the present study were younger and smaller than African American peers from NHANES III (Table 2). Malagasy children showed the lowest stature and BMI, and they had a relatively larger trunk, as indicated by the highest Cormic index (Table 1, Figure E1).

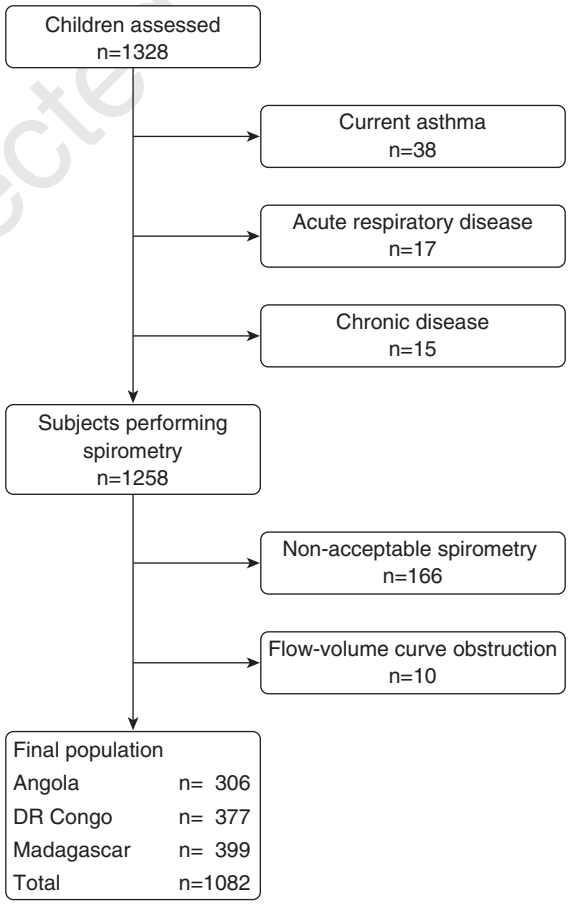

Figure 1. Study population. Urban and semiurban healthy children (6–12 yr) from public and private primary schools in Angola, Democratic Republic of the Congo (DR Congo), and Madagascar were included.

Table 1. Population Characteristics and Lung Function in Children from Angola, Democratic Republic of the Congo, and Madagascar

	Angola (*n* = 306)	DR Congo (*n* = 377)	Madagascar (*n* = 399)
Boys, %	50	55	51
In public school, n (% boys)	94 (50)	191 (53)	147 (50)
Age, yr	9.8 (1.9)	9.5 (1.6)	8.7 (1.5)
Sitting/standing height	0.48 (0.04)*	0.50 (0.02)*	0.52 (0.02)*
zHeight†	−0.28 (1.30)*	0.32 (1.30)*	−1.22 (1.13)*
zBMI‡	−0.64 (2.16)*	−0.20 (1.10)*	−1.07 (1.08)*
$zFEV_1$	−0.32 (0.74)*	−0.16 (0.79)*	0.10 (0.88)*
zFVC	−0.38 (0.80)*	−0.09 (0.83)*	0.16 (0.84)*
$zFEV_1/FVC$	0.10 (0.78) *	−0.17 (0.71)	−0.10 (0.95)

Definition of abbreviations: BMI = body mass index; DR Congo = Democratic Republic of the Congo; zBMI = *z*-score for BMI; $zFEV_1$ = *z*-score for FEV_1; zFVC = *z*-score for FVC; $zFEV_1/FVC$ = *z*-score for FEV_1/FVC; zHeight = *z*-score for height.
Results are presented as mean (SD) unless otherwise specified. Spirometry *z*-scores based on Global Lung Function Initiative–2012 equations for African Americans (1).
*Differences between countries: $P < 0.001$(analysis of variance and Tukey's honestly significant difference test).
†zHeight values based on World Health Organization growth charts (40).
‡zBMI values based on CDC growth charts (39).

This correlated negatively with zBMI ($P < 0.01$). Thinness was observed in 15.5% and stunted growth (zHeight < -2) in 11.6% of sub-Saharan children; 2.5% combined stunted growth and thinness (Figure E2). There were large differences in nutritional status between individuals in Angola, as evidenced by the large SD for zBMI (Table 1).

Fit to GLI-2012 Reference and Comparison with NHANES III Peers

After adjusting for sex, age, and height using GLI-2012 black equations, mean (SD) *z*-scores in collated data of African children were −0.11 (0.83) for FEV_1, −0.08 (0.86) for FVC, and −0.07 (0.83) for FEV_1/FVC. When compared with black American counterparts from NHANES III, the FEV_1 and FVC but not the FEV_1/FVC ratio in African children were slightly but significantly lower; however, in normotrophic African pupils (n = 914), neither these indices nor the Cormic index differed from those in NHANES III (*t* test, *P* from 0.057 to 0.192; Table 2, Figure 2). Thin children (zBMI < -2) from Angola, DR Congo, and Madagascar (n = 168) had significant reductions of ~0.5 *z*-scores in both FEV_1 and FVC (with preserved FEV_1/FVC) compared with African American peers from NHANES III (Table 2, Figure 2).

Table 2. Anthropometric *z*-Scores and Lung Function in African American Children and sub-Saharan African Peers from Angola, Democratic Republic of the Congo, and Madagascar

		African Children	
	African Americans NHANES III Study (*n* = 837)	Normotrophic (*n* = 914)	Thin (zBMI < −2) (*n* = 168)
Boys, %	51	53	49
Age, yr	10.4 (1.3)	9.3 (1.7)*	9.3 (1.8)*
zHeight†	0.71 (1.50)	−0.39 (1.40)*	−0.66 (1.40)*
zBMI‡	—	−0.21 (1.11)	−3.01 (1.22)
Cormic index	0.50 (0.014)	0.50 (0.030)	0.48 (0.040)*
$zFEV_1$	0.03 (1.06)	−0.04 (0.83)	−0.46 (0.76)*
zFVC	0.08 (1.05)	−0.01 (0.85)	−0.49 (0.73)*
$zFEV_1/FVC$	−0.07 (1.12)	−0.09 (0.83)	0.05 (0.86)

Definition of abbreviations: BMI = body mass index; NHANES = National Health and Nutrition Examination Survey; zBMI = *z*-score for BMI; $zFEV_1$ = *z*-score for FEV_1; zFVC = *z*-score for FVC; $zFEV_1/FVC$ = *z*-score for FEV_1/FVC; zHeight = *z*-score for height.
Results are presented as mean (SD), unless otherwise specified. Spirometry *z*-scores based on Global Lung Function Initiative–2012 equations for African Americans (1).
*Difference relative to African American children: $P < 0.001$ (analysis of variance and Tukey's honestly significant difference test).
†zHeight values based on World Health Organization growth charts (40).
‡zBMI values based on CDC growth charts (39).

Comparison of the Three African Countries

There was wide overlap of spirometric data (Figure 3) with a slight negative trend of *z*-scores with age (lowest $P = 0.015$, Figure 3) for FEV_1 and FVC in Angolan and Congolese children; the FEV_1/FVC ratios in the three countries were unrelated to age (lowest $P = 0.153$). The scatter in the *z*-scores (indicated by the SD) of all spirometric indices was appreciably smaller than 1, pointing to great homogeneity in lung function in each of the populations (Table 1). Due to the small SD, the fifth centile for spirometric indices in the collated data was not at a *z*-score of −1.64, but at −1.30 in boys and −1.50 in girls. After adjusting for sex, age, and height, FEV_1 and FVC were largest in Malagasy and lowest in Angolan children (Table 1). The *z*-scores for FEV_1 and FVC differed systematically between countries (AOV, $P < 0.0001$). The *z*-scores for FVC and FEV_1/FVC were marginally smaller in public than in private school children ($P = 0.035$, explained variance 0.3%, data not shown).

Spirometric Outcomes in Relation to Body Proportions in African Children

Cormic index contributed significantly ($P < 0.03$ in boys, $P < 0.0001$ in girls) to explaining differences in FEV_1 and FVC in African children. *Z*-scores for FEV_1 and FVC correlated positively with zBMI ($P < 0.0001$) but not with the Cormic index ($P > 0.09$). Disparities in nutritional status contributed 1.3 to −1.6% per unit of zBMI to between-subject differences in FEV_1 and FVC (Table 3). Thin African children (zBMI < -2) had a 4.5% lower FEV_1 (−0.41 *z*-score units) and 5.4% lower FVC (−0.49 *z*-score units) than normotrophic ones ($P < 0.0001$), but the FEV_1/FVC ratio

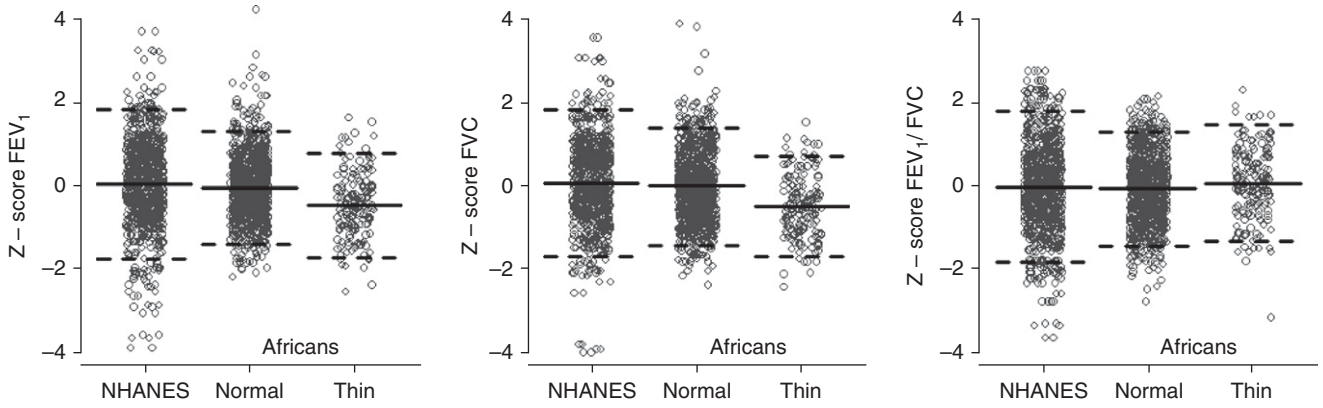

Figure 2. Spirometry data, adjusted for sex, age, and height according to Global Lung Function Initiative 2012 equations for African Americans (1), in black American children from the NHANES (National Health and Nutrition Examination Survey) III study and children from Angola, Democratic Republic of the Congo, and Madagascar (Africans). *Solid lines* indicate mean values. *Dashed lines* delineate the 90% confidence intervals. Thin African children had a BMI *z*-score < -2 according to CDC growth charts (39).

did not differ between these two groups ($P = 0.06$) (Table 2, Figure 2).

Discussion

This is the first study assessing the applicability of GLI-2012 reference values for African Americans in children and adolescents from various sub-Saharan countries. It also evaluated the effects of impaired nutritional status on lung function. The findings confirm previous observations that differences in FEV_1 and FVC between groups of healthy subjects are proportional, as evidenced by a comparable FEV_1/FVC ratio (1, 20–22, 47–49). This can be expected, because humans have the same lung design, so that in healthy subjects differences in lung volumes reflect differences in chest size (50). Conventionally, stature is used as a proxy for lung volumes, but this does not consider diversity in body frame. This is illustrated

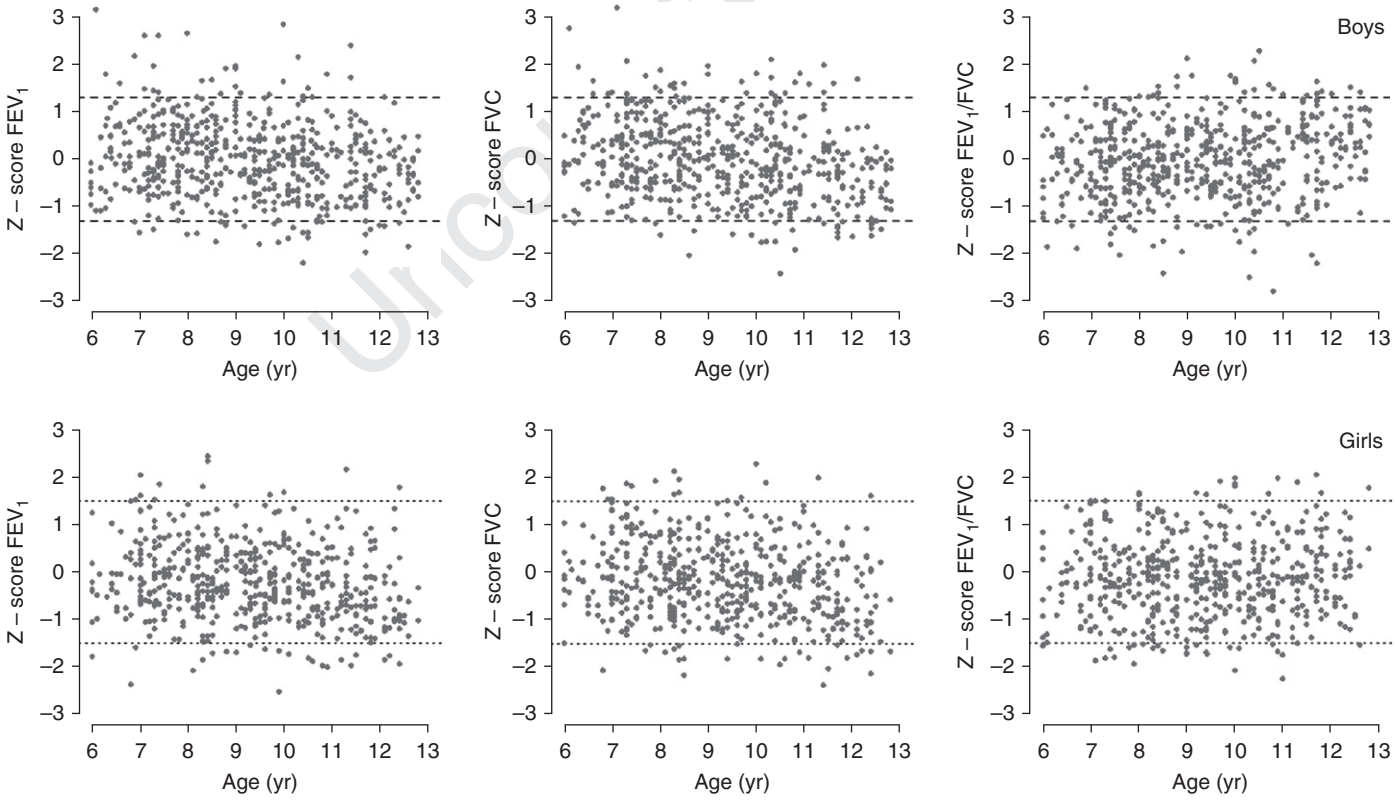

Figure 3. Distribution of *z*-scores for FEV_1, FVC, and the FEV_1/FVC ratio as a function of age in boys (*upper* panels) and girls (*lower* panels) applying Global Lung Function Initiative 2012 predicted values for African Americans. *Dashed lines* indicate the fifth and 95th centiles at ±1.64 *z*-scores.

Table 3. Regression Coefficients of Relationship between FEV_1 and FVC and Explanatory Variables, Including Country of Residence (Angola as Reference), in sub-Saharan African Children

	Boys (*n* = 552)		Girls (*n* = 530)	
	Estimate	***P* Value**	**Estimate**	***P* Value**
ln(FEV_1)				
Intercept	−10.1164	<0.0001	−9.9448	<0.0001
ln(Height)	2.1293	<0.0001	2.0656	<0.0001
Age	0.0129	0.0021	0.0201	<0.0001
DR Congo	−0.0033	0.7744	0.0216	0.0727
Madagascar	0.0312	0.0081	0.0527	<0.0001
zBMI	0.0142	<0.0001	0.0125	<0.0001
ln(FVC)				
Intercept	−10.4813	<0.0001	−10.4753	<0.0001
ln(Height)	2.2289	<0.0001	2.1996	<0.0001
Age	0.0124	0.0029	0.0182	0.0002
DR Congo	0.0189	0.0931	0.0353	0.031
Madagascar	0.0463	<0.0001	0.0667	<0.0001
zBMI	0.0157	<0.0001	0.0138	<0.0001

Definition of abbreviations: BMI = body mass index; DR Congo = Democratic Republic of the Congo; ln = natural logarithm; zBMI = *z*-score for BMI.
Differences in zBMI contribute 1.3 to −1.6% per unit zBMI to differences in FEV_1 and FVC between subjects.

by the biologically plausible pattern shown in this study where disparities in body frame affect the comparisons between African countries. Children with the lowest BMI were shorter and had relatively shorter legs (i.e., larger Cormic index), hallmarks of adverse conditions during childhood (26). Their FEV_1 and FVC were proportionally smaller, as shown by a normal FEV_1/FVC ratio (Figure 2); this implies that there is no respiratory impairment but that growth retardation leads to smaller chest dimensions, which are not properly accounted for by stature as a proxy for lung size. This study showed that this even holds true in the case of overt malnutrition.

There were small, albeit statistically significant, differences in lung function indices between different African countries. Differences of the magnitude found in this study may occur if the sample size is small (43). Although ethnic heterogeneity might also play a role, this is unlikely for children from Angola and DR Congo. Both countries are mainly inhabited by Bantu-speaking groups who migrated from grass field regions between Cameroon and Nigeria around 5,000 years ago (34). Studies on uniparentally transmitted mitochondrial DNA and Y-chromosome variation in these populations show that they are genetically quite homogeneous, with little genetic mixing with other groups (34, 35). The Malagasy population comprises a mix of people of Austronesian ancestry, who inhabit mainly the central highlands, and Bantu immigrants from East Africa, who prevail in the coastal area (36, 37). We studied children from the coastal area, but some genetic Bantu-Austronesian mixing is present in all Malagasy ethnic groups (37). Children from Madagascar had the highest Cormic index (Table 1), and, in spite of heterogenic ethnic background, they show the best fit to GLI-2012 black prediction equations (Table 1). A recent study (11) demonstrated that predicted values for African Americans fit the adult Austronesian inhabitants of Madagascar. This study provides further evidence that the GLI-2012 predicted values are applicable in subjects with predominant Bantu ancestry across sub-Saharan Africa. Overall, in view of the widely overlapping distributions (Figure 3), it seems justified to collate the data from the three countries.

Lung Function in African versus African American Children and the Impact of Malnutrition

After excluding African children with poor nutritional status (zBMI < -2), spirometry outcomes in the African children are fully comparable to those in African American peers and fit GLI-2012 predicted values. Our findings are consistent with some previous reports in adults (10, 11) and children (12) from sub-Saharan Africa, which found comparable dynamic lung volumes in healthy African and black American subjects. In view of the large differences in average level of affluence, healthcare access, and exposure to air pollution between African American children and their peers in sub-Saharan Africa, the results of this study highlight the predominant role of genetic background rather than environment in determining lung function (27). The gross domestic product converted to international dollars using purchasing power parity rates in DR Congo is 1.5%, in Madagascar 2.6%, and in Angola 14.5% of that in the United States (51), with income being very unevenly distributed. The poor conditions are reflected in the large percentages of thin children (Table 2, Figure E2) with low stature and low leg length, hallmarks of insufficient growth (26). Growth retardation is associated with smaller chest dimensions, as indicated by the proportionally reduced FEV_1 and FVC. Recently, Sonnappa and colleagues (21) made similar observations. They found that spirometric outcomes in UK-Indian children were very similar to those in well-nourished urban Indian children, whereas the semiurban and rural Indians had significant but proportional reductions of FEV_1 and FVC, associated with a much lower BMI. It follows that well-nourished children with common genetic ancestry living in developing and developed countries appear to have comparable lung function.

z-Scores, which indicate how many SDs a measurement differs from the predicted value, used in this study, have the advantage that they are free of bias related to height, age, sex, and ethnicity, unlike percent predicted. In the case of a perfect fit of predicted values to a dataset, the standard deviation of *z*-scores should be one unit. In the studied children, it is consistently smaller (Table 1), indicating that there is greater homogeneity in this population than in African American boys and girls from the NHANES III study, in whom there is greater variability than expected (i.e., SD of spirometric *z*-scores > 1, Table 2). Unlike in Africa, there is considerable mixture of African and European genes in African Americans (13, 14), which might explain these striking differences.

A limitation of this study is that it included only schoolchildren. Each of these countries has public and private schools, but many parents cannot afford the school fees,

leading to school attendance rates as low as 60%, with many children not completing primary school and also some overrepresentation of boys (52–54). Therefore, this study has not covered the full spectrum of SES in these countries. Children not attending school belong to the poorest segment of the population and are at higher risk of impaired nutritional status and exposure to indoor air pollution, factors that can affect lung function. Another limitation is that we could not investigate the level of exposure to outdoor and indoor pollution and how this affected spirometry outcomes. The smaller scatter (i.e., between-person variability) in the present study and associated shift of the lower limit of normal above the fifth centile compared with GLI-2012 may be a chance finding due to limited sample size (43) and needs confirmation in other sub-Saharan African populations.

A strength of this study is that data collection was performed in three countries by the PI, applying the same selection criteria, using identical equipment, and adopting the same quality control procedures, in accordance with ATS/ERS recommendations. In addition, the study covers the full age range of primary school in these countries.

Future Directions

To create robust spirometry prediction equations for all sub-Saharan African populations, future studies comprising at least 300 healthy male and female subjects (43) should explore the applicability of GLI-2012 predicted values in other African countries. Studies should preferably cover the entire age range and look into the role of SES and indoor and outdoor air pollution on lung function. Malnutrition and stunting affect somatic development and hence the relationship between stature and lung volumes; they should therefore be taken into account. The measurement of sitting and standing height may be useful for detecting differences in body frame that might cause differences in pulmonary function between ethnic groups.

Conclusions

GLI-2012 reference values for spirometry are appropriate for healthy, well-nourished African children attending school in Angola, DR Congo, and Madagascar, but the lower limit of normal may need adjustment. Lung function in well-nourished African pupils was fully comparable to that of African American peers from NHANES III. Growth retardation due to malnourishment accounts for smaller but normally developed lungs in healthy children. In well-nourished children, genetic background predominates over environment in determining lung function. ■

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References

1. Quanjer PH, Stanojevic S, Cole TJ, Baur X, Hall GL, Culver BH, Enright PL, Hankinson JL, Ip MS, Zheng J, *et al*.; ERS Global Lung Function Initiative. Multi-ethnic reference values for spirometry for the 3-95-yr age range: the global lung function 2012 equations. *Eur Respir J* 2012; 40:1324–1343.
2. Binder RE, Mitchell CA, Schoenberg JB, Bouhuys A. Lung function among black and white children. *Am Rev Respir Dis* 1976;114: 955–959.
3. Schwartz J, Katz SA, Fegley RW, Tockman MS. Sex and race differences in the development of lung function. *Am Rev Respir Dis* 1988;138:1415–1421.
4. Hankinson JL, Odencrantz JR, Fedan KB. Spirometric reference values from a sample of the general U.S. population. *Am J Respir Crit Care Med* 1999;159:179–187.
5. Eveleth PB, Tanner JM. Worldwide variation in human growth. Cambridge: Cambridge University Press; 1990.
6. Brehm JM, Acosta-Pérez E, Klei L, Roeder K, Barmada MM, Boutaoui N, Forno E, Cloutier MM, Datta S, Kelly R, *et al*. African ancestry and lung function in Puerto Rican children. *J Allergy Clin Immunol* 2012;129: 1484–1490.e6.
7. Menezes AM, Wehrmeister FC, Hartwig FP, Perez-Padilla R, Gigante DP, Barros FC, Oliveira IO, Ferreira GD, Horta BL. African ancestry, lung function and the effect of genetics. *Eur Respir J* 2015;45:1582–1589.
8. Chan Y, Salem RM, Hsu YH, McMahon G, Pers TH, Vedantam S, Esko T, Guo MH, Lim ET, Franke L, *et al*.; GIANT Consortium. Genome-wide analysis of body proportion classifies height-associated variants by mechanism of action and implicates genes important for skeletal development. *Am J Hum Genet* 2015;96:695–708.
9. Ben Saad H, El Attar MN, Hadj Mabrouk K, Ben Abdelaziz A, Abdelghani A, Bousarssar M, Limam K, Maatoug C, Bouslah H, Charrada A, *et al*. The recent multi-ethnic global lung initiative 2012 (GLI2012) reference values don't reflect contemporary adult's North African spirometry. *Respir Med* 2013;107:2000–2008.
10. Musafiri S, van Meerbeeck JP, Musango L, Derom E, Brusselle G, Joos G, Rutayisire C. Spirometric reference values for an East-African population. *Respiration* 2013;85:297–304.
11. Ratomaharo J, Linares Perdomo O, Collingridge DS, Andriamihaja R, Hegewald M, Jensen RL, Hankinson J, Morris AH. Spirometric reference values for Malagasy adults aged 18-73 years. *Eur Respir J* 2015;45:1046–1054.
12. Glew RH, Kassam H, Vander Voort J, Agaba PA, Harkins M, VanderJagt DJ. Comparison of pulmonary function between children living in rural and urban areas in northern Nigeria. *J Trop Pediatr* 2004;50:209–216.
13. Bryc K, Auton A, Nelson MR, Oksenberg JR, Hauser SL, Williams S, Froment A, Bodo JM, Wambebe C, Tishkoff SA, *et al*. Genome-wide patterns of population structure and admixture in West Africans and African Americans. *Proc Natl Acad Sci USA* 2010;107:786–791.
14. Kumar R, Seibold MA, Aldrich MC, Williams LK, Reiner AP, Colangelo L, Galanter J, Gignoux C, Hu D, Sen S, *et al*. Genetic ancestry in lung-function predictions. *N Engl J Med* 2010;363:321–330.
15. Wolff PT, 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. *Pediatr Pulmonol* 2014; 49:76–83.
16. Harik-Khan RI, Fleg JL, Muller DC, Wise RA. The effect of anthropometric and socioeconomic factors on the racial difference in lung function. *Am J Respir Crit Care Med* 2001;164:1647–1654.
17. Harik-Khan RI, Muller DC, Wise RA. Racial difference in lung function in African-American and White children: effect of anthropometric, socioeconomic, nutritional, and environmental factors. *Am J Epidemiol* 2004;160:893–900.
18. Whitrow MJ, Harding S. Ethnic differences in adolescent lung function: anthropometric, socioeconomic, and psychosocial factors. *Am J Respir Crit Care Med* 2008;177:1262–1267.
19. Ong TJ, Mehta A, Ogston S, Mukhopadhyay S. Prediction of lung function in the inadequately nourished. *Arch Dis Child* 1998;79:18–21.
20. Faridi MM, Gupta P, Prakash A. Lung functions in malnourished children aged five to eleven years. *Indian Pediatr* 1995;32:35–42.

21. Sonnappa S, Lum S, Kirkby J, Bonner R, Wade A, Subramanya V, Lakshman PT, Rajan B, Nooyi SC, Stocks J. Disparities in pulmonary function in healthy children across the Indian urban-rural continuum. *Am J Respir Crit Care Med* 2015;191:79–86.
22. Raju PS, Prasad KVV, Ramana YV, Balakrishna N, Murthy KJR. Influence of socioeconomic status on lung function and prediction equations in Indian children. *Pediatr Pulmonol* 2005;39:528–536.
23. Lum S, Bountziouka V, Sonnappa S, Wade A, Cole TJ, Harding S, Wells JC, Griffiths C, Treleaven P, Bonner R, *et al*. Lung function in children in relation to ethnicity, physique and socioeconomic factors. *Eur Respir J* 2015;46:1662–1671.
24. Strippoli M-PF, Kuehni CE, Dogaru CM, Spycher BD, McNally T, Silverman M, Beardsmore CS. Etiology of ethnic differences in childhood spirometry. *Pediatrics* 2013;131:e1842–e1849.
25. Ware JH, Dockery DW, Spiro A III, Speizer FE, Ferris BG Jr. Passive smoking, gas cooking, and respiratory health of children living in six cities. *Am Rev Respir Dis* 1984;129:366–374.
26. Cole TJ. Secular trends in growth. *Proc Nutr Soc* 2000;59:317–324.
27. Quanjer PH. Lung function, genetics and socioeconomic conditions. *Eur Respir J* 2015;45:1529–1533.
28. Kurmi OP, Lam KBH, Ayres JG. Indoor air pollution and the lung in low- and medium-income countries. *Eur Respir J* 2012;40:239–254.
29. World Health Organization. Country profiles of environmental burden of disease [accessed 2016 Jul 20]. Available from: http://www.who.int/quantifying_ehimpacts/national/countryprofile/en/
30. Gauderman WJ, Avol E, Gilliland F, Vora H, Thomas D, Berhane K, McConnell R, Kuenzli N, Lurmann F, Rappaport E, *et al*. The effect of air pollution on lung development from 10 to 18 years of age. *N Engl J Med* 2004;351:1057–1067.
31. Centers for Disease Control and Prevention. National Health and Nutrition Examination Survey: NHANES III (1988-1994) – data files [accessed ■■■]. Available from: http://www.cdc.gov/nchs/nhanes/nh3data.htm
32. Arigliani M, Canciani MC, Magnolato A, Quanjer PH. Normal lung function in Angolan children [abstract]. *Eur Respir J* 2015;46:A1272.
33. Arigliani M, Canciani MC, Altomare M, Bonetti MA, Currò P, Dadic M, Mottini G, Quanjer PH. Lung function and nutritional status in urban Malagasy children [abstract]. *Eur Respir J* 2014;44:A192.
34. de Filippo C, Barbieri C, Whitten M, Mpoloka SW, Gunnarsdóttir ED, Bostoen K, Nyambe T, Beyer K, Schreiber H, de Knijff P, *et al*. Y-chromosomal variation in sub-Saharan Africa: insights into the history of Niger-Congo groups. *Mol Biol Evol* 2011;28:1255–1269.
35. Barbieri C, Vicente M, Oliveira S, Bostoen K, Rocha J, Stoneking M, Pakendorf B. Migration and interaction in a contact zone: mtDNA variation among Bantu-speakers in Southern Africa. *Plos One* 2014; 9:e99117.
36. Hurles ME, Sykes BC, Jobling MA, Forster P. The dual origin of the Malagasy in Island Southeast Asia and East Africa: evidence from maternal and paternal lineages. *Am J Hum Genet* 2005;76:894–901.
37. Tofanelli S, Bertoncini S, Castrì L, Luiselli D, Calafell F, Donati G, Paoli G. On the origins and admixture of Malagasy: new evidence from high-resolution analyses of paternal and maternal lineages. *Mol Biol Evol* 2009;26:2109–2124.
38. Asher MI, Keil U, Anderson HR, Beasley R, Crane J, Martinez F, Mitchell EA, Pearce N, Sibbald B, Stewart AW, *et al*. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. *Eur Respir J* 1995;8:483–491.
39. Centers for Disease Control and Prevention. Percentile data files with LMS values. 2009 [accessed 2016 Jul 25]. Available from: http://www.cdc.gov/growthcharts/percentile_data_files.htm
40. World Health Organization. BMI-for-age (5-19 years) [accessed 2016 Jul 25]. Available from: http://www.who.int/growthref/who2007_bmi_for_age/en/
41. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, Crapo R, Enright P, van der Grinten CP, Gustafsson P, *et al*.; ATS/ERS Task Force. Standardisation of spirometry. *Eur Respir J* 2005;26:319–338.
42. Kirkby J, Welsh L, Lum S, Fawke J, Rowell V, Thomas S, Marlow N, Stocks J; EPICure Study Group. The EPICure study: comparison of pediatric spirometry in community and laboratory settings. *Pediatr Pulmonol* 2008;43:1233–1241.
43. Quanjer PH, Stocks J, Cole TJ, Hall GL, Stanojevic S; Global Lungs Initiative. Influence of secular trends and sample size on reference equations for lung function tests. *Eur Respir J* 2011;37:658–664.
44. Rigby RA, Stasinopoulos DM. Generalized additive models for location, scale and shape. *J R Stat Soc Ser C Appl Stat* 2005;54:507–554.
45. Cole TJ, Stanojevic S, Stocks J, Coates AL, Hankinson JL, Wade AM. Age- and size-related reference ranges: a case study of spirometry through childhood and adulthood. *Stat Med* 2009;28:880–898.
46. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc B* 1995;57:289–300.
47. Rossiter CE, Weill H. Ethnic differences in lung function: evidence for proportional differences. *Int J Epidemiol* 1974;3:55–61.
48. Gray LA, Leyland AH, Benzeval M, Watt GCM. Explaining the social patterning of lung function in adulthood at different ages: the roles of childhood precursors, health behaviours and environmental factors. *J Epidemiol Community Health* 2013;67:905–911.
49. Korotzer B, Ong S, Hansen JE. Ethnic differences in pulmonary function in healthy nonsmoking Asian-Americans and European-Americans. *Am J Respir Crit Care Med* 2000;161:1101–1108.
50. Paul R, Fletcher GH, Addison G. A comparative study between Europeans and Africans in the mining industry of Northern Rhodesia. *Med Proc (Johannesb)* 1960;6:69–74.
51. The World Bank. GDP per capita, PPP (current international $) [accessed ■■■]. Available from: http://data.worldbank.org/indicator/NY.GDP.PCAP.PP.CD
52. UNICEF. Madagascar: education [accessed 2016 Jul 25]. Available from: http://www.unicef.org/madagascar/5559.html
53. UNICEF. Angola: statistics [accessed 2016 Jul 25]. Available from: http://www.unicef.org/infobycountry/angola_statistics.html
54. Education Policy and Data Center. ■■■ [accessed 2016 Jul 25]. Available from: http://www.epdc.org/sites/default/files/documents/congodemrep_coreusaid.pdf

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