Biceps Skin-fold Thickness May Detect and Predict Early Lipoatrophy in HIV-infected Children

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Background: The prevalence of potentially stigmatizing lipoatrophy in children receiving antiretroviral therapy in Southern Africa is high, affecting around a third of children. Early diagnosis of lipoatrophy is essential for effective intervention to arrest progression.

Methods: Prepubertal children receiving antiretroviral therapy were recruited from a hospital-based family HIV clinic in Cape Town and followed up prospectively. Lipoatrophy was identified and graded by consensus between 2 HIV pediatricians. A dietician performed anthropometric measurements of trunk and limb fat. Anthropometric measurements in children with and without lipoatrophy were compared using multivariable linear regression adjusting for age and gender. The most discerning anthropometric indicators of lipoatrophy underwent receiver operating characteristic curve analysis. The precision of anthropometric measurements performed by an inexperienced healthcare worker was compared with that of a research dietician.

Results: Of 100 recruits, 36 had lipoatrophy at baseline and a further 9 developed lipoatrophy by 15-month follow-up. Annual incidence of lipoatrophy was 12% (confidence interval [CI]: 5–20%) per person-year of follow-up. A biceps skin-fold thickness <5 mm at baseline had a sensitivity of 89% (CI: 67–100%) and a specificity of 60% (CI: 46–75%) for predicting development of lipoatrophy by 15-month follow-up. Negative and positive predictive values were 97% (CI: 91–100%) and 32% (CI: 14–50%).

Conclusion: Biceps skin-fold thickness <5 mm in prepubertal children exposed to thymidine analogue-based antiretroviral therapy may be a useful screening tool to identify children who are likely to develop lipoatrophy. The variation in precision of measurements performed by an inexperienced healthcare worker only marginally impacted performance.

Key Words: lipoatrophy, lipodystrophy, screening, biceps, skin-fold thickness, prepubertal, children, antiretroviral therapy, South Africa, HIV

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Long-term use of antiretroviral therapy (ART), particularly the nucleoside reverse transcriptase inhibitors stavudine and zidovudine, may result in disfiguring loss of subcutaneous fat, termed lipoatrophy.[1] Although programmatic changes are starting to phase out stavudine use, stavudine remains the most commonly used antiretroviral for HIV-infected children in sub-Saharan Africa.[1,2] Even in South Africa, while children initiating ART after 2010 have been initiated on abacavir, current guidelines state that children taking stavudine should continue unless side effects develop, at which stage the child should be switched to abacavir.[3] In most other sub-Saharan African countries, the cost of abacavir remains prohibitive and the most common alternative is zidovudine, which also causes lipoatrophy albeit less severely.[4,5]

The prevalence of lipoatrophy in children on ART appears to be rising over time. Studies from 2005 and 2006 estimated the prevalence of lipoatrophy at 8% to 11%[6,7] whereas the most recent studies have found a prevalence of around 28%.[8] Accumulation of cases is not surprising because lipoatrophy changes may persist

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despite switching of ART regimen, and survival rates are high in medication-adherent children who are at highest risk of lipoatrophy. Recently we have shown that the prevalence of lipoatrophy in prepubertal African children on any ART in South Africa is 36%.[9]

Diagnosis of early lipoatrophy is difficult. Although an objective case definition for lipoatrophy has been established for adults,[10] this has not been validated in children and the current standard for diagnosing lipoatrophy in children is the skilled visual assessment of subcutaneous limb and face fat performed by experienced pediatric HIV clinicians who have been specifically trained to do this.[11] In developed countries, serial magnetic resonance imaging and serial dual-energy radiography absorptiometry scanning are available to monitor the amount and distribution of subcutaneous fat in HIV-infected children exposed to thymidine analogue ART. Radiographic methods are not feasible in resource-constrained settings, and pediatric-trained HIV specialists are scarce. A simple screening tool is needed to detect lipoatrophy in prepubertal children before it causes stigmatizing disfigurement, allowing appropriate antiretroviral switches to be made early to arrest progression. The tool should require minimal equipment or training and should be easy for primary care nurses and ancillary healthcare workers to use. The aim of the current study was to develop an anthropometric screening tool to detect lipoatrophy in prepubertal children. We performed a second study to test the impact of imprecision in anthropometric measurements performed by an inexperienced healthcare worker on the diagnostic performance of the screening tool.

METHODS

The Family Clinic for HIV at Tygerberg Children's Hospital is a public sector clinic providing ART to infants and children from the northern suburbs of Cape Town. In this prospective study, children who were 3–12 years old on ART and prepubertal were recruited between February 2010 and January 2011. Prepubertal status was defined as Tanner stage 1 for genital and pubic hair development in boys, and Tanner stage 1 for pubic hair development with Tanner stage 1 or 2 for breast development in girls. Inclusion of girls with Tanner stage 2 breast development was allowed in this study because early minimal thelarche is not uncommon in very young girls in whom it does not typically herald the onset of true puberty.[12,13] Using review of our electronic health record database, we identified 190 patients who potentially met inclusion criteria. Of these, 124 attended clinic during the study period and could be approached for screening. Of 121 who agreed to participate, 21 did not attend the study visit nor did they respond to attempts at further contact. There was no difference in demographic characteristics of the 100 enrolled subjects and the 90 who were not recruited ($P >$ 0.20 for age, gender, cumulative stavudine exposure and CD4, data not shown).

Lipoatrophy was identified and graded by consensus between 2 experienced HIV pediatricians using the following lipoatrophy grading scale as defined by existing literature[10,14,15]: 0 – No fat changes; 1 – Possible minor changes, noticeable only on close inspection; 2 – Moderate changes, readily noticeable to an experienced clinician or a close relative who knows the child well; 3 – Major changes, readily noticeable to a casual observer. Face, arms, legs and buttocks were assessed for loss of subcutaneous fat resulting in abnormally prominent limb veins, a lean, muscular appearance of limbs and face, and loss of gluteal fat pad with reduction in buttock size and loss of gluteal contour. Where the assessments of the 2 investigators did not concur; the change was graded as the lower score. Lipoatrophy was defined as a score of 2 or 3. Lipoatrophy assessment was repeated at 15-month follow-up. The duration and details of prior ART and demographics were recorded from our electronic health record database. HIV RNA values and CD4 values were extracted from our central electronic laboratory results server. Doses of antiretroviral drugs followed nationally prescribed protocols. For stavudine this meant a minimum of 1 mg/kg twice daily rounded up to the nearest practical dose.[16] A professional dietician performed formal dietary assessment and anthropometric measurements of trunk and limb fat using a nonstretchable tape-measure (model number F10-02DM, Muratec KDS Corporation, Kyoto, Japan), a high-precision Harpenden skin-fold caliper (Baty International, West Sussex, United Kingdom), a ShorrBoard stadiometer (Shorr Productions, MD) and a precision weighing scale (model number UC-321, A&D Company, Tokyo, Japan), which was calibrated daily. The stated accuracy of the Harpenden skin-fold caliper is 99%, with a dial graduation of 0.2 mm and repeatability of 0.2 mm.[17] Measurements included mid-upper arm circumference, mid-thigh circumference, chest circumference, waist circumference, hip circumference, biceps skin-fold thickness (SFT), triceps SFT, iliac crest SFT, subscapular SFT, mid-thigh SFT, height and weight. All anthropometric measurements were performed 3 times and averaged. The following ratios were derived: waist-to-hip circumference ratio; body mass index; torso-to-arm SFT ratio ([subscapular + iliac crest SFT] / [biceps + triceps SFT]); waist-to-mid-upper arm circumference ratio and weight-to-mid-upper arm circumference ratio. Diet assessment variables included in the analysis were total daily carbohydrate consumption, total daily fat consumption and total daily calorie consumption. The dietician graded each as inadequate, appropriate or excessive. Data were stored in a secure electronic database using REDCap software (https://redcap.vanderbilt.edu, Vanderbilt University, Nashville, TN).

Analysis

Baseline data were compared between patients with and without lipoatrophy using *t* test for continuous variables and chi-square test for categorical variables. Univariate Spearman's correlation analysis was performed between maximum lipoatrophy grading score and each of the anthropometric measures. To adjust for age and gender, multiple linear regression models were conducted to assess the associations between each of the anthropometric measures and 4-point lipoatrophy grading scores. SFT data were log-transformed for analysis. The model used the anthropometric measure as the dependent variable and lipoatrophy grading score, age and gender as the independent variables. Partial Spearman's correlations were calculated between the 4-point lipoatrophy score and each of the anthropometric measures adjusted for age and gender, using the variance–covariance matrix.

Receiver operating characteristic (ROC) curve analysis was performed on anthropomorphic variables that were significant in the multiple regression analyses. ROC analyses (using pROC package in R statistical software, The R Foundation for Statistical Computing, Vienna, Austria) were conducted to compare the performance of these anthropometric measures in predicting lipoatrophy. Two sets of analyses were performed. The first used baseline anthropometric measures to predict baseline prevalent lipoatrophy diagnosis. The second used baseline anthropometric measures to predict the 1-year incident lipoatrophy outcome. The latter analysis only included patients without lipoatrophy at baseline. For each variable, a threshold was determined at which the values of sensitivity and specificity for detecting early lipoatrophy were optimized. Positive predictive value (PPV) and negative predictive value (NPV) were calculated. For each of these anthropometric measures, empirical ROC curves and partial area under the curve (pAUC; between 80% and 100% sensitivity) with correction

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by McClish[18] were calculated. 95% confidence intervals (CIs) were determined by bootstrap for ROC AUC, sensitivity and specificity, and by Gaussian approximation for NPV and PPV. Statistical analyses were performed using R version 2.10.0 (Bell Laboratories, NJ).

Second Study to Determine the Precision of Anthropometric Measurements Performed by an Inexperienced Observer

In a second study, anthropometric measurements performed by an inexperienced observer (an early-stage medical student) were compared with measurements performed by a highly skilled and experienced research dietician. Before the study began, the inexperienced observer received basic instruction on the anthropometric method for each measurement. Measurements were then performed separately by the inexperienced observer and the research dietician on the same children on the same day using the same equipment used in the parent study, without observation or communication between the research dietician and the inexperienced observer. Coefficients of variation were calculated. For each anthropometric variable, the difference between the inexperienced observer's measurement and the research dietician's measurement were calculated. This difference was then incorporated into the threshold anthropometric values used by the screening tool, in order to assess how the difference in precision might impact the effectiveness of the screening tool.

This study was designed in accordance with the guidelines of the International Conference on Harmonization for Good Clinical Practice and with the Declaration of Helsinki (version 2000) and approved and monitored by the Ethics Committee for Human Research of the Stellenbosch University, approval reference number N08/11/349. Written informed consent was obtained from each caregiver before participation, and informed assent was obtained from capable children.

RESULTS

Thirty-six children had lipoatrophy at baseline and 64 did not. Baseline characteristics are presented in Table 1. World Health Organization clinical stage, CD4, HIV RNA values and formal dietary assessment variables were similar between those with and without lipoatrophy. One of the 15 girls with lipoatrophy and 2 of the 33 girls without lipoatrophy were Tanner stage 2 for breast development at recruitment. All others were Tanner stage 1. Twenty-nine of the 36 children with lipoatrophy at baseline had been diagnosed with lipoatrophy before enrolment, of whom 23 had been switched to abacavir >6 months before assessment. Lipoatrophy grading scores at baseline and at follow-up are presented in Table 2 and Figure 1.

Baseline anthropomorphic measures (mean ± standard deviation) that were significantly different ($P < 0.001$) between those with and those without lipoatrophy were biceps SFT (3.8 ± 1.1 versus 5.3 ± 2.2), triceps SFT (6.6 ± 1.9 versus 9.0 ± 3.0) and torso-to-arm SFT ratio (1.1 ± 0.3 versus 0.9 ± 0.3) whereas body mass index Z score and waist-to-hip circumference ratio were not different between groups. Separate multivariable models, using the 3 significant SFT measures as the dependent variables and the full 4-point lipoatrophy score, age and sex as independent variables, showed significant correlations between SFT and lipoatrophy score after adjusting for age and gender. When adjusted for age and gender, partial correlation coefficients between the 3 significant SFT measures and 4-point lipoatrophy score were 0.33 (biceps, $P = 0.0006$), 0.37 (triceps, $P = 0.0001$) and 0.39 (torso-to-arm ratio, $P = 0.0001$).

Partial ROC AUC for 80%–100% sensitivity, calculated to determine the optimal differentiating anthropometric variable, was nominally higher for biceps (0.7; 95% CI: 0.59–0.80) than for triceps or torso-to-arm ratio SFT (0.68 and 0.66, respectively). These are shown in Figure 2, n = 97, where the horizontal light

TABLE 1. Comparison of HIV-infected Children With and Without Visually Obvious Lipoatrophy

	Children With Lipoatrophy N = 36	Children Without Lipoatrophy N = 64	Univariate *P* (2-tailed)
Median age at ART initiation, with IQR	24 (9–43)	19 (9–37)	0.74
Median age at recruitment (mo) (IQR)	89 (71–112)	71 (50–92)	0.001
Gender: male/female	21 (58%) / 15 (42%)	31 (48%) / 33 (52%)	0.41
Median nadir absolute CD4 before ART initiation (IQR)	694 (439–798)	802 (447–1131)	0.29
Median nadir CD4% before ART initiation (IQR)	15% (7–21%)	17% (14–25%)	0.05
Absolute CD4 at recruitment (IQR)	1213 (919–1556)	1129 (792–1524)	0.57
CD4% at recruitment (IQR)	37% (30–40%)	31% (26–37%)	0.03
Median $\log_{10}$ viral load at recruitment (IQR)	1.85 (1.60–2.54)	1.85 (1.60–2.54)	0.10
Number with HIV RNA viral load <400 copies/mL	35 (97%)	55 (86%)	0.07
Maximum World Health Organization clinical stage ever reached: 1 / 2 / 3 / 4	25% / 11% / 39% / 25%	17% / 9% / 46% / 28%	0.78
Median weight for age Z score (IQR)	–1.0 (–1.8 to –0.5)	–1.0 (–1.6 to –0.3)	0.39
Median height for age Z score (IQR)	–1.1 (–2.0 to –0.5)	–1.3 (–2.3 to –0.8)	0.49
Median body mass index Z score (IQR)	–0.6 (–1.1 to 0.0)	–0.2 (–0.8 to 0.6)	0.008
Number on second-line therapy, defined as switch of ≥2 antiretroviral drugs (%)	3 (8%)	4 (6%)	0.58
Any antiretroviral exposure, median months (IQR)	56 (44–75)	43 (25–60)	0.002
Number ever exposed to stavudine (%)	35 (97%)	53 (83%)	0.04
Stavudine, median months (IQR)	41 (27–48)	30 (7–49)	0.02
Number ever exposed to zidovudine (%)	17 (47%)	16 (25%)	0.02
Zidovudine, median months (IQR)	12 (5–21)	0 (0–0)*	0.56
Lamivudine, median months (IQR)	52 (41–72)	41 (25–58)	0.01
Number ever exposed to lopinavir/r (%)	22 (61%)	50 (78%)	0.07
Lopinavir/r, median months (IQR)	26 (0–56)	36 (6–51)	0.58
Number ever exposed to efavirenz (%)	17 (47%)	19 (30%)	0.09
Efavirenz, median months (IQR)	0 (0–44)*	0 (0–4)*	0.003

*Because fewer than half of the subjects in these groups had been exposed to these drugs, the median exposure was 0 months. Mean efavirenz exposure was 23 months in children with lipoatrophy versus 7 months in children without lipoatrophy. Mean zidovudine exposure was 8 months in children with lipoatrophy versus 10 months in children without lipoatrophy.

IQR indicates interquartile range.

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TABLE 2. Lipoatrophy Grading Scores at Baseline and at Follow-up

Baseline		Follow-up	
Maximum Lipoatrophy Score	n	Maximum Lipoatrophy Score	n
0	58	0	39
		1	11
		2	5
		3	0
1	6	0	1
		1	0
		2	3
		3	1
2	24	0	2
		1	3
		2	13
		3	4
3	12	0	0
		1	0
		2	6
		3	6

grey shape corresponds to the pAUC region. The pAUC of biceps SFT with 95% CI is printed in the middle of the plot. The best threshold of 4.8 mm with corresponding specificity (60.7%) and sensitivity (88.9%) for biceps SFT is also located in the plot. The total ROC area under curve value was 0.75 (CI: 0.64–0.84). As the biceps measurement is also clinically easier to perform, this was selected as the optimal measure. The biceps SFT ROC curve revealed that the threshold value for biceps SFT that gave optimal screening sensitivity and specificity for differentiating children with and without lipoatrophy at baseline was 4.85 mm. A clinically measurable biceps SFT threshold of 5 mm had a sensitivity of 89% (CI: 78–97%) and a specificity of 61% (CI: 48–72%) to detect lipoatrophy at baseline. NPV was 90% (CI: 80–99%) and PPV was 57% (CI: 42–68%).

Follow-up was completed on 34 of the 36 children with lipoatrophy and 60 of the 64 children without lipoatrophy at baseline. Median follow-up time was 14.9 months (interquartile range: 14.5–15.6 months). At follow-up, 9 of the 60 children had developed new lipoatrophy, giving an incidence rate of 12% (CI: 5–20%) per person-year of follow-up. Lipoatrophy had resolved in 5 of 34 children, all of whom had been switched to abacavir >18 months before and had no more than moderate (grade 2) lipoatrophy signs at baseline.

In children without lipoatrophy at baseline, ROC analysis of baseline biceps SFT revealed an area under curve value of 0.83 (CI: 0.67–1.00) and a partial ROC area under curve value for sensitivity between 80% and 100% of 0.69 (CI: 0.54–0.93) for predicting which children would go on to develop lipoatrophy by 15-month follow-up (Fig. 3). In comparison, the ability of triceps SFT to predict lipoatrophy at follow-up was limited, with a partial ROC area under curve value for sensitivity between 80% and 100% of 0.56 (CI: 0.46–0.91). Figure 1 shows ROC curves of biceps SFT, triceps SFT and torso-to-arm SFT ratio to predict new lipoatrophy at follow-up. In children without lipoatrophy at baseline, a baseline biceps SFT <5 mm yielded a sensitivity of 89% (CI: 67–100%) and a specificity of 60% (CI: 46–75%) for predicting which children would go on to develop lipoatrophy. NPV was 97% (CI: 91–100%) and PPV was 32% (14–50%). Posttest probabilities were as follows: In the presence of a negative test, the posttest probability of developing lipoatrophy by 15-month follow-up was 0.10 (CI: 0.02–0.18), whereas the probability of not developing lipoatrophy was 0.91 (CI: 0.84–0.99). In the presence of a positive test, the posttest probability of developing lipoatrophy by 15-month follow-up was 0.59 (CI: 0.46–0.71), whereas the probability of not developing lipoatrophy was 0.41 (CI: 0.29–0.54). Repeating the analyses without the 3 children who had Tanner stage 2 breast development at baseline marginally improved the performance of the screening tool both at baseline and at follow-up. Proportions of ethnic sub-groups were similar between those with and without lipoatrophy ($P > 0.30$).

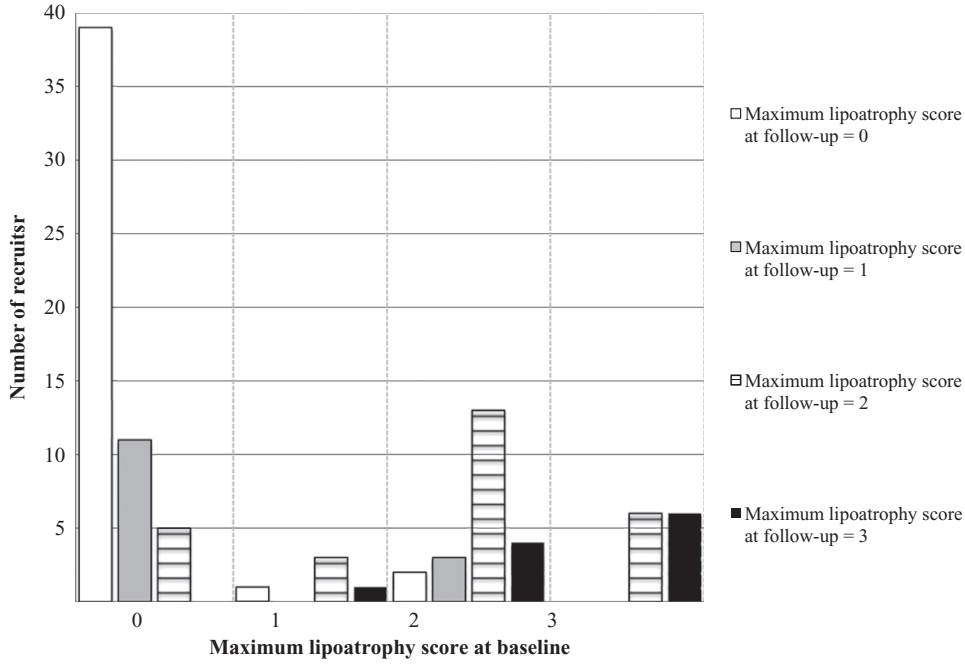

FIGURE 1. Lipoatrophy grading scores at baseline and at follow-up.

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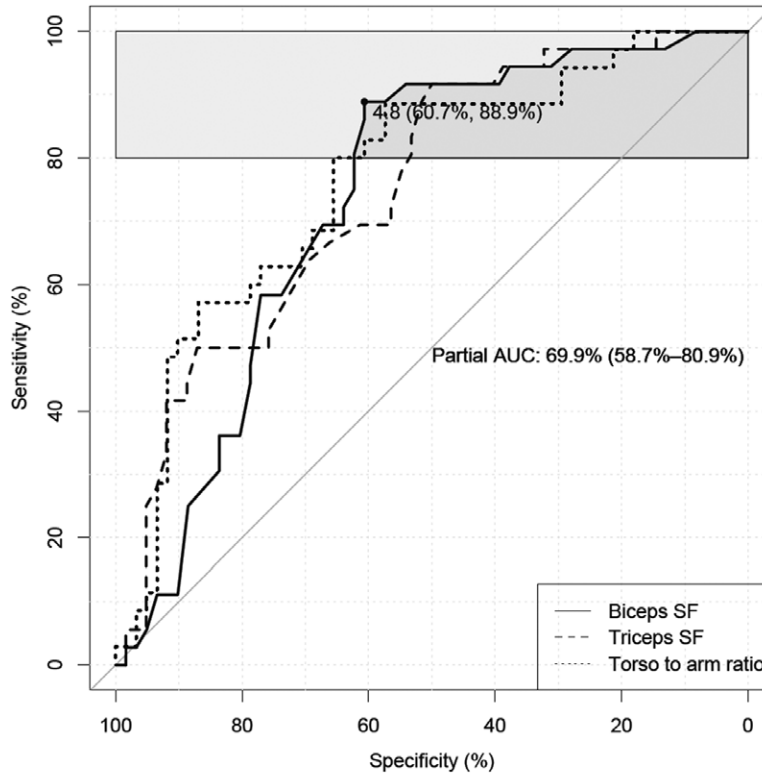

ROC = Receiver Operating Characteristic curve; SFT = skin-fold thickness;
pAUC = partial area-under-the-curve for sensitivity between 80% and 100%.

FIGURE 2. ROC curves of biceps SFT (solid line), triceps SFT (dashed line) and torso-to-arm SFT ratio (dotted line) to detect prevalent lipoatrophy at baseline, n = 97. The horizontal light grey shape corresponds to the pAUC region. The pAUC of biceps SFT with 95% confidence interval is printed in the middle of the plot. The best threshold of 4.8 mm with corresponding specificity (60.7%) and sensitivity (88.9%) for biceps SFT is also located in the plot.

Second Study to Determine the Precision of Anthropometric Measurements Performed by an Inexperienced Observer

An additional 77 children from the same clinic were recruited between April and June 2009 to compare the precision of anthropometric measurements performed by an inexperienced observer to that of an experienced research dietician. These subjects had a median age of 3.0 years (interquartile range 2.0–3.8 years) and 37 (48%) were female. Two subsequently refused to cooperate with any measurements and 6 refused SFT measurements. SFT measurements were successfully completed on 69 children. The measured differences between the inexperienced observer and the research dietician are presented in Table 3. The mean absolute difference in biceps SFT measurement between the inexperienced observer and the research dietician was 0.8 mm. Taking this variability into account, the sensitivity, specificity, PPV and NPV of biceps SFT thresholds of <4 mm and <6 mm were calculated in order to determine how the variability in precision might affect the performance of the screening tool for predicting which children would have lipoatrophy at 15-month follow-up (Table 4). The variation in precision of measurements performed by an inexperienced healthcare worker only marginally impacted performance.

Each anthropometric measurement had been performed 3 times and averaged. The mean absolute difference between the 3 values, calculated as the mean of the differences between the first and second value, the second and third value, and the first and third value, was used to calculate the intraobserver variability for the inexperienced observer compared to the experienced research dietician (Table 5). The intraobserver variability of the inexperienced observer compared favorably to that of the experienced research dietician.

DISCUSSION

Lipoatrophy looks very similar to AIDS wasting syndrome, termed "Slims disease" throughout Africa, and may confer the same stigmatization. In contrast to the developed world, stigmatization due to HIV in the communal cultures of sub-Saharan Africa may lead to loss of housing, loss of employment or livelihood, denial of schooling, denial of healthcare, secondary stigmatization of family members and physical violence.[19,20] Patients who develop recognizable ART-related fat distribution abnormalities may become nonadherent to ART in order to avoid stigmatization,[21,22] which will result in declining CD4 cells, development of opportunistic infections and possibly death. This is particularly true of adolescents who are piquantly concerned about body image and social acceptance, and who may become nonadherent even in the absence of ART-related body changes.[23] In previous reports, partial recovery occurred in the least severely affected individuals.[24,25] Severe lipoatrophy may not be reversible.[25–27] This is understandable because lipoatrophy is due to progressive apoptosis of adipocytes, which do not recover,[28]

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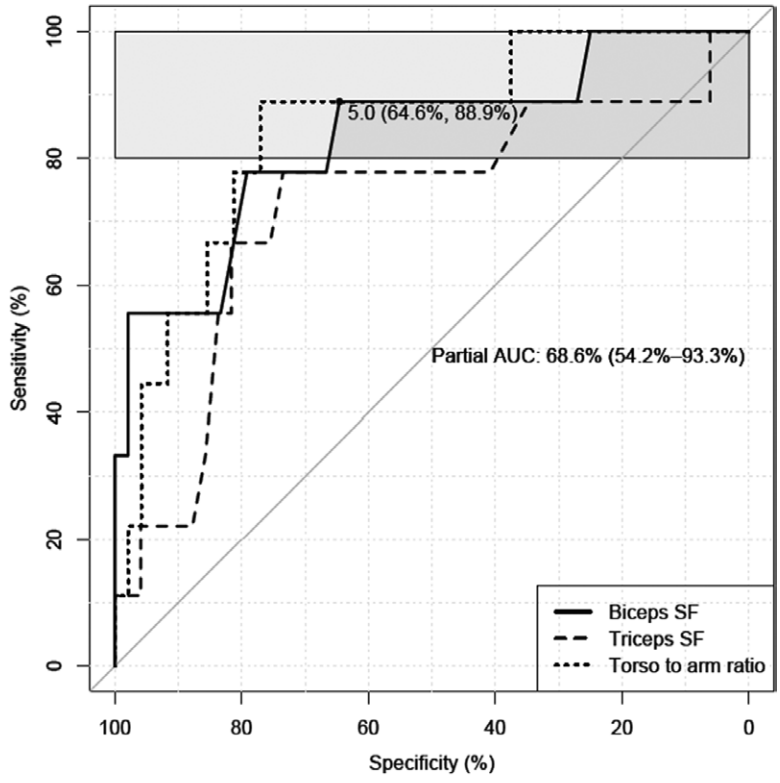

ROC = Receiver Operating Characteristic curve; SFT = skin-fold thickness; pAUC = partial area-under-the-curve for sensitivity between 80% and 100%.

FIGURE 3. ROC curves of biceps SFT (solid line), triceps SFT (dashed line) and torso-to-arm SFT ratio (dotted line) to predict which children will go on to develop new lipoatrophy by 15-month follow-up, n = 58. The horizontal light grey shape corresponds to the pAUC region. The pAUC of biceps SFT with 95% confidence interval is printed in the middle of the plot. The best threshold of 5 mm with corresponding specificity (64.6%) and sensitivity (88.9%) for biceps SFT is also located in the plot.

as opposed to nutritional wasting where adipocyte fat stores shrink but the cell survives. However, stigmatization only occurs when lipoatrophy is easily recognizable by the broader community. Communities with the highest prevalence of HIV are the most likely to recognize HIV-related or ART-related body changes. Southern Africa has the highest HIV prevalence in the world.[29] Stigmatization can be prevented if lipoatrophy is diagnosed early and appropriate ART switches are made, which will arrest lipoatrophy progression.[27]

Children with and without lipoatrophy had similar immunologic and clinical presentation at ART initiation and at recruitment ($P > 0.2$ for absolute CD4 and World Health Organization clinical staging). They did not start ART earlier and a similar proportion was on second-line ART. The median age of children with lipoatrophy was higher than that of children without lipoatrophy (7.4 versus 5.9 years, $P = 0.001$). This difference in age was expected because the risk of lipoatrophy increases with cumulative ART use,[9] which increases with age.

TABLE 3. Precision of Anthropometric Measurements Performed by an Inexperienced Observer Compared With an Experienced Research Dietician (Interobserver Variability)

N = 69	Measurements Performed by Inexperienced Observer			Measurements Performed by Experienced Research Dietician			Measured Pairwise Absolute Difference Between Inexperienced Observer and Research Dietician	
	Mean	SD	CV (%)	Mean	SD	CV (%)	Mean	SD
Waist circumference (cm)	50.4	3.9	8%	50.1	3.3	7%	0.9	0.9
Hip circumference (cm)	49.7	3.8	8%	49.1	7.6	15%	1.7	1.5
Mid-upper arm circumference (cm)	15.7	1.8	11%	15.9	1.3	8%	0.5	0.4
Triceps SFT (mm)	9.1	3.4	38%	9.2	2.2	24%	0.9	0.9
Biceps SFT (mm)	5.9	3.8	64%	6.0	1.5	26%	0.8	0.6
Subscapular SFT (mm)	6.5	4.1	64%	6.3	1.7	27%	0.5	0.4
Iliac crest SFT (mm)	8.2	4.5	54%	6.7	2.9	44%	1.7	1.5

CV indicates coefficient of variation; SD, standard deviation.

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TABLE 4. Effectiveness of Biceps SFT Threshold of <5 mm With Variations in Precision of ±1 mm, for Predicting Any Lipoatrophy at 15-month Follow-up (Incorporating Resolved Cases, Persistent Cases and New Cases), n = 94

Biceps SFT Threshold	Sensitivity	Specificity	PPV	Negative Predictive Value
<4 mm	68%	75%	65%	78%
<5 mm	89%	63%	62%	90%
<6 mm	95%	43%	53%	92%

Some partial improvement of lipoatrophy may be experienced after drug switching, with the more severe cases experiencing the least improvement.[24] The biceps SFT at baseline was less informative for detecting existing cases than for predicting new cases at follow-up. Individuals known to have lipoatrophy before recruitment had been switched to abacavir months or years before recruitment and may have experienced some improvement in subcutaneous SFT before recruitment as a result of the switch. This may explain the limited performance of the screening tool in detecting lipoatrophy at baseline. The ROC analysis for predicting new cases at follow-up may be more helpful because new cases at follow-up had not been influenced by interventional drug switches.

The ROC analyses at baseline did not confirm that any of the 3 SFT contenders (biceps, triceps and arm-to-trunk ratio) was statistically superior to the others. However, the biceps SFT technique is the most simple to perform, and biceps SFT measurements had the least inter- and intraobserver variability. These strengths led us to choose biceps SFT over triceps SFT and torso-to-arm SFT ratio. Using a threshold of 5 mm for biceps SFT yields a high sensitivity and NPV, making it effective for screening. The relatively low specificity is clinically acceptable; as the next step after screening is referral, not change of therapy. The screening tool can be employed by nurses and ancillary healthcare workers who can then refer children with suspected early lipoatrophy to pediatric HIV doctors for confirmation and antiretroviral switching as necessary. Experienced HIV pediatricians who have been specifically trained to identify early lipoatrophy will typically use the formal visual grading scale described above to confirm the diagnosis.

Lipoatrophy does not occur in all children exposed to ART.[8,11,14] Some show no signs of fat changes despite many years of stavudine or zidovudine exposure, whereas others develop lipoatrophy within 18 months of ART initiation.[25] This variation may be due to genetic differences that increase or decrease an individual's susceptibility to lipoatrophy,[30–32] which would explain why viral load did not clearly differentiate between children with and without lipoatrophy in our study. Ethnic differences may also play a role.[8] In the face of this unpredictability, our screening tool provides an objective, easy-to-use method to identify children who may be developing lipoatrophy.

The current studies were performed using a Harpenden caliper, which although highly precise, is expensive ($340). A Slimguide skin-fold caliper (Rosscraft, Vancouver, Canada) is marginally less precise (repeatability 0.5 mm)[33] but is inexpensive ($20) and durable, and may be a more suitable device to roll out in resource-limited primary healthcare settings in sub-Saharan Africa. The reason for using the more precise Harpenden caliper rather than the Slimguide in our second study was to isolate and quantify the imprecision of measurements performed by an inexperienced healthcare worker, and to be consistent with the parent study. Before implementation of the Slimguide can be recommended, a further study is needed in which repeated measurements are performed by an experienced anthropometric dietician using both the Harpenden and Slimguide calipers to quantify the additional imprecision contributed by using the less precise device.

For broad implementation of this screening tool, a brief written description of how to perform a biceps SFT measurement may be all the training that is necessary. For children on ART, the effort required to perform a single annual biceps SFT measurement is likely to be logistically feasible even in busy community healthcare clinics. Serial annual biceps SFT measurements also give the added benefit of identifying changes from baseline: A biceps SFT that has dropped from a higher baseline to below 5 mm, in the absence of an obvious nutritional or other cause, is probably a more convincing indication of impending lipoatrophy than a static measurement below 5 mm.

Inclusion of girls with Tanner stage 2 breast development was allowed in this study because early minimal thelarche is not uncommon in very young girls in whom it does not typically herald the onset of true puberty.[12,13] However, since the presence of early breast tissue may be associated with a higher body fat proportion, the analyses were repeated without the children with stage 2 breast development. Only 3 recruits had Tanner stage 2 breast development at baseline, and repeating the analyses without the 3 children in fact marginally improved

Our data on the use of biceps SFT with a cutoff of 5 mm as a screening tool for lipoatrophy has a number of limitations: These findings apply only to pre-pubertal children between 3 and 12 years of age. While this period of life has relatively little fluctuation in SFT and body fat distribution, age and gender do have some impact on global subcutaneous fat and biceps SFT. Our limited sample size did not allow stratification of ROC cut-off thresholds by age or gender. It is possible that the most appropriate diagnostic cut-off value may be different for younger versus older children and for boys versus girls. Converting biceps SFT to an age- and gender-related percentile

TABLE 5. Intraobserver Variability of the Inexperienced Observer Compared With the Experienced Research Dietician, Using Mean Absolute Difference in Repeated Measurements

N = 69	Inexperienced Observer Measurements			Experienced Research Dietician Measurements		
	Mean	SD	CV (%)	Mean	SD	CV (%)
Waist circumference (cm)	0.49	0.48	97%	0.44	0.38	87%
Hip circumference (cm)	0.33	0.27	82%	0.34	0.32	95%
Mid-upper arm circumference (cm)	0.13	0.11	84%	0.13	0.10	78%
Triceps SFT (mm)	0.42	0.33	79%	0.38	0.38	102%
Biceps SFT (mm)	0.29	0.26	90%	0.24	0.26	106%
Subscapular SFT (mm)	0.25	0.22	86%	0.17	0.16	92%
Iliac crest SFT (mm)	0.41	0.30	74%	0.30	0.33	107%

CV indicates coefficient of variation; SD, standard deviation.

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would adjust for these differences, however, calculation and interpretation of percentiles is complex, particularly for the minimally-trained primary care nurses and ancillary healthcare workers for whom the screening tool is intended. In addition, population reference charts for biceps SFT in African children 3 to 12 years of age have not previously been compiled and building percentile charts de novo would be difficult. Change in biceps SFT over time from each individual's own baseline may be a more useful marker of impending lipoatrophy, and analysis of longitudinal data from this cohort is underway. It is important for clinicians to keep in mind that the onset of puberty will substantially alter subcutaneous fat amount and distribution due to the influence of sex hormones, and an absolute value is unlikely to be useful in that setting. For pubertal children, a change in biceps SFT percentile could be investigated as a more appropriate indicator of impending lipoatrophy. While biceps SFT is a reasonable surrogate measure of global subcutaneous fat, this variable does have a non-Gaussian population distribution, with percentile lines above the median being more widely spaced than percentile lines below the median. This suggests that biceps SFT is more variable in fatter children than thinner children. In addition, the accuracy of SFT callipers is reduced at higher readings. However, lipoatrophy is typically a condition of thinner children in whom these limitations have the least impact. The current study had a limited sample size, resulting in broad CIs and possibly misleading results. A follow-up study is needed to test the performance of this screening tool on a different cohort of prepubertal HIV-infected African children on ART. The standard deviation for SFT measurements performed by the experienced research dietician was up to 4 times that of previously published findings.[34] This raises concern about the precision of those measurements, which were used as a reference for measurements performed by the inexperienced healthcare worker. The imprecision may have produced misleading results, which could have altered the assessment of the reliability of the screening tool in inexperienced hands. The multivariable analyses demonstrated a significant effect of age and sex on the various anthropomorphic measures; the study's small sample size did not allow for stratification of ROC cutoff thresholds by age and sex. Thus, a measure of 5 mm may have a different prognosis in a younger versus older child. The very high negative predictive value of the 5 mm cutoff is reassuring that few cases would be missed despite the lack of age-specific cutoffs. The low specificity may lead to a significant number of unnecessary referrals, which may overburden secondary referral services. Precision of biceps skin-fold thickness is technique dependent, which requires some training. An inconsistent method of measurement may lead to increased intraobserver variability. Differences in method between healthcare workers may lead to increased interobserver variability. Both intra- and interobserver variability may have the consequence that a patient's change in biceps skin-fold thickness from baseline may go unnoticed. Although durable, if the Slimguide caliper is used, the spring strength will eventually deteriorate, which may not be obvious and may lead to continued use when the caliper should be replaced, resulting in inaccurate and possibly misleading measurements. Reliance on an objective measurement may lead to reduced clinical vigilance on the part of primary healthcare staff, particularly in busy clinics where patient burdens are large. The high sensitivity may falsely reassure healthcare workers, leading to missed cases. This screening tool should not replace diligent attentiveness during routine follow-up. Specificity and PPV are limited and confirmation of suspected lipoatrophy using visual grading assessment by a skilled operator remains necessary. Adherence was not measured or correlated with lipoatrophy, and this was a weakness of the current study.

In conclusion, a biceps SFT of <5 mm in HIV-infected prepubertal children exposed to thymidine analogue-based ART may be an objective, relatively easy-to-use screening tool to identify children who currently have or may progress to develop lipoatrophy, allowing appropriate antiretroviral drug switches to be made at an early stage, which will arrest progression and avoid stigmatizing disfigurement.

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