Author’s response to reviews

Title: High prevalence of lipoatrophy in pre-pubertal South African children on antiretroviral therapy: A cross-sectional study

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Author’s response to reviews: see over
Dear Mr Atienza and Dr John,

Thank you for your reviewers' helpful comments. Below is a point-by-point response to each.

Reviewer 1 (Dr Thanyawee Puthanakit)

Abstract

- In the abstract's results section, the sentence "51/64 (80%) of children without lipoatrophy were on stavudine-based regimens at recruitment" has been removed as suggested, as we recognize the potential for confusion.
- The statement "Adjusted odds ratio for developing lipoatrophy was 1.9 (95% CI: 1.3 - 2.9) for each additional year of accumulated exposure to standard dose stavudine" has been added to the abstract as suggested.

Background

- The connection between stavudine and lipoatrophy is well established. Literature to this effect has been quoted in the seventh paragraph of the discussion.
- We have added the following paragraph to the discussion comparing the strength of a visual grading scale as primary outcome measure over anthropometry or DXA in the sub-Saharan African context: “Although DXA and anthropometry may be more precise measures of subcutaneous fat amount and distribution, a visual grading scale was chosen as the primary outcome measure in this study because the greatest danger of lipoatrophy in sub-Saharan Africa stems from stigmatization. Subtle changes in fat distribution that are not visually obvious are less relevant since they are unlikely to result in stigmatization.”

Method

- The following inclusion criteria statement has been repeated in the methods section after the description of the clinic setting in which recruitment took place: “In this cross-sectional study, children who were 3-12 years old, on antiretroviral therapy and pre-pubertal were recruited. Pre-pubertal status was determined using Tanner staging.”
- We have added the following statement to the methods section as requested: “The sites inspected were face, arms, legs and buttocks.”
- As requested, we have clarified that “additional normative data was collected from healthy age-, gender- and socioeconomically-matched HIV-uninfected children from the same community who had been enrolled as part of a different study at our research unit.” In addition we have clarified that “this local normative data appears in tables 2 and 3 to assist the reader to gauge the magnitude of the changes found in lipoatrophy-affected children.”

Results

- The reviewer stated that “No information of overall subject baseline characteristics was given”. We refer the reader to table 1, which is referenced in the third sentence of the results section.
This provides detailed baseline immunological, clinical, demographic and growth data for both lipoatrophy-affected and -unaffected children.

- As requested, we have added information about the doses of antiretroviral drugs, and specifically stated that “Doses of antiretroviral drugs followed nationally prescribed protocols. For stavudine this meant a minimum of 1mg/kg twice daily rounded up to the nearest practical dose.”

- We recognize the confusion that may be caused by stating that 19% of children who developed lipoatrophy had been exposed to stavudine in the past 6 months compared to 71% of children who did not develop lipoatrophy. We have therefore removed the last line of table 1 and added the following statement to the results section: “At the time of assessment, 29/36 children with lipoatrophy had been off stavudine for at least six months with no resolution of their symptoms.”

- As requested we have clarified that “Where the assessment of the two investigators did not concur, the change was graded as 1. Lipoatrophy was defined as a score of 2 or 3.”

- The p-values in tables 2 and 3 were the adjusted ANOVA p-values. However, after consideration of the reviewer’s comment, we have instead presented the p-value specifically comparing HIV-infected children with and without lipoatrophy. We have added a statement to this effect to the captions of tables 2 and 3.

- Our purpose in showing anthropometric and DXA data was to present an objective verification of the subjective visual lipoatrophy grading score. As requested, a formal analysis of the correlation between these has been added. We have presented the results in the form of an adjusted correlation coefficient for each of the anthropometric and DXA variables when compared to visual lipoatrophy grading score, adjusted for age and gender.

Discussion

- The third paragraph of the discussion intends to point out that sub-Saharan African populations are different to developed world populations and need to be studied specifically. The similarities between the African and Asian context have been added in the fourth paragraph as follows: “This finding is in line with that of the Asian cohort reported by Aurpibul et al, which found an increasing prevalence of fat distribution abnormalities as cumulative exposure to ART increased. This is significant since Asian cohorts have similar conditions to sub-Saharan Africa in that malnutrition is common, access to ART for children is incomplete, and stavudine has been the most widely used first-line antiretroviral agent.”

Reviewer 2 (Dr Leah Kern)

Minor essential revisions

1. This has been altered as suggested.
2. This has been altered as suggested.
3. This has been altered as suggested.
4. This has been altered as suggested.
5. This has been altered as suggested.

Discretionary revisions

1. This has been altered as suggested.
2. In the abstract’s results section, the sentence “51/64 (80%) of children without lipoatrophy were on stavudine-based regimens at recruitment” has been removed as suggested. The statement “Adjusted odds ratio for developing lipoatrophy was 1.9 (95% CI: 1.3 - 2.9) for each additional year of accumulated exposure to standard dose stavudine” has been added to the abstract as suggested.
3. The following inclusion criteria statement has been added to the methods section after the description of clinic setting in which recruitment took place: “In this cross-sectional study, children who were 3-12 years old, on antiretroviral therapy and pre-pubertal were recruited. Pre-pubertal status was determined using Tanner staging.”

4. We have added the phrase “who were experienced in identifying lipoatrophy” as advised. As requested we have clarified that “Where the assessment of the two investigators did not concur, the change was graded as 1. Lipoatrophy was defined as a score of 2 or 3.”

5. The following clarification has been added: “Durations of previous antiretroviral exposures and demographics were recorded from our electronic health record database. HIV RNA and CD4 values were extracted from our central electronic laboratory results server.”

6. We have added the following sentence to the methods section to describe the children who underwent DXA scanning: “DXA was requested for all recruits, however, as DXA is a rare commodity in the developing world, DXA was not always available. There was no difference in gender, cumulative time on standard dose stavudine or CD4 between the 42 subjects who underwent DXA scanning and the 58 who did not (p=0.50 for all). The children who underwent DXA were marginally younger than those who did not (7.1 versus 8.0 years, p=0.03). In addition, we have added the following clarification of which DXA measurements were performed: “Trunk and limb fat mass, lean mass and fat percentage were measured.”

7. We have added the following sentence to clarify the reason for this: “Zidovudine and didanosine were not included in the analysis as too few children had been exposed to these drugs.”

8. We have corrected this sentence to read: “All but one of the children with lipoatrophy had been exposed to more than 18 months of stavudine therapy.”

9. We have added the following sentence to address this issue: “In our context, specific training to recognize lipoatrophy in children typically includes didactic training followed by supervised practice in a clinic setting over a period of time until competence is reached to the satisfaction of the trainer.”

10. This is a valid point. Diagnosis of early lipoatrophy using visual grading requires training and experience. We have altered this conclusion to read: “A simple objective screening tool is needed that can identify early lipoatrophy in resource-limited settings where specialized skills and equipment are not available.”

Reviewer 3 (Dr Takara Stanley)
Minor essential revisions

1. In the abstract’s results section, the sentence “51/64 (80%) of children without lipoatrophy were on stavudine-based regimens at recruitment” has been removed as suggested. The statement “Adjusted odds ratio for developing lipoatrophy was 1.9 (95% CI: 1.3 - 2.9) for each additional year of accumulated exposure to standard dose stavudine” has been added to the abstract as suggested.

2. The following has been added to the methods section as advised: “In univariate analyses, a t-test was used for parametric data, Wilcoxon rank sum test was used for non-parametric data, and Fisher’s exact test was used for categorical data. Non-parametric data is quoted as median (interquartile range), and parametric data is quoted as mean (standard deviation) or
mean (95% confidence interval) where appropriate” and “A least squares approach was used for multivariate modeling.”

3. We have altered this sentence to read: “This is most likely due to higher rates of stavudine exposure in our context. The magnitude of this difference suggests that data from immigrant African populations in Europe cannot be extrapolated to populations living in sub-Saharan Africa”

4. As suggested, we have moved the paragraph describing prevalence data in earlier studies to before the discussion of Alam’s data in the third paragraph of the discussion section.

Discretionary revisions

1. The following sentence has been added to the abstract as suggested: “Cumulative time on standard dose stavudine was significantly associated with reductions in biceps and triceps skin-fold thickness (p=0.008).”

2. Thank you for the suggestion. In the cross-sectional study by Wohl et al of 737 HIV-infected men and 145 HIV-uninfected matched controls from the FRAM study, peripheral lipoatrophy measured by MRI was associated with significantly higher serum triglyceride concentration (170 vs 107mg/dL, p<0.0001) after adjustment for central fat accumulation. In the cross-sectional study by Lake et al of 519 HIV-infected and 260 HIV-uninfected matched controls from the same FRAM study, peripheral lipoatrophy measured by MRI was associated with significant increase in 10-year Framingham Risk Score for incident cardiovascular events (4.7% vs 3.7%, p=0.0002) after adjustment for central fat accumulation but not for pre-existing cardiovascular disease, smoking or HDL cholesterol, all of which differed significantly between groups on univariate analysis (4% vs 0%, p=0.009; 45% vs 21%, p<0.0001; and 38 vs 46mg/dL, p<0.0001 respectively). We are not aware of published prospective data linking lipoatrophy per se to increased cardiovascular risk and since the discussion around that topic remains inconclusive we prefer not to include it in our introduction.

3. We have replaced BMI with BMI Z-scores in table 1 as suggested.

4. We recognize the confusion that may be caused by stating that 19% of children who developed lipoatrophy had exposed to stavudine in the past 6 months compared to 71% of children who did not develop lipoatrophy. We have therefore removed the last line of table 1 and added the following statement to the results section: “At the time of assessment, 29/36 children with lipoatrophy had been off stavudine for at least six months with no resolution of their symptoms.”

The conflict of interest statement has been moved from the foot of the title page to immediately following the conclusion as requested.

The following additional changes have been made:

- The number of children on ART in South Africa has been updated in the second paragraph of the Background section.
- “Cumulative stavudine exposure” has been reworded as “cumulative time on standard dose stavudine”. We believe this clarification is important since exposure to a lower dose may have different metabolic consequences. Lowered-dose stavudine is likely to become standard-of-care in areas where pediatric alternatives to stavudine are not yet reliably available.
- The p-value comparing demographic characteristics of the 100 enrolled subjects and the 90 who were not recruited has been corrected in the methods section.
- Since skin-fold thickness is in fact a log-normal variable, we have redone the analyses using log-transformed skin-fold thickness data.

Best regards

Steve Innes

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