Author’s response to reviews

Title: Lipid and glucose alterations in perinatally-acquired HIV-infected adolescents and young adults.

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Author’s response to reviews: see over
Dear Dr. Torti,

We would like to thank the reviewers for their insightful and constructive review of our manuscript entitled “Lipid and glucose alterations in perinatally-acquired HIV-infected adolescents and young adults”.

We have amended the manuscript and addressed all reviewer comments as described in the enclosed table and in the accompanying manuscript.

We believe the revised manuscript addresses the reviewers’ concerns and queries, and that these revisions have strengthened the manuscript. We hope you find the revised manuscript suitable for publication in BMC Infectious Diseases and look forward to your decision regarding final acceptance and publication of our work.

Sincerely,
Daniel Blázquez, MD.

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<th>REVIEWER'S COMMENTS</th>
<th>AUTHOR’S RESPONSE</th>
<th>REFERENCE PAGES</th>
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<tr>
<td><strong>Reviewer 1:</strong> Nadir CD4 was associated with insulin resistance on univariate analysis, but it seems that this variable was not adjusted in multivariate analysis.</td>
<td>Nadir CD4 is now included in the multivariate logistic regression analysis model. New adjusted result for abdominal circumference is included in results section, and nadir CD4 is listed in table 3.</td>
<td>Methods section: Lines 194-197 Table 3.</td>
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<td><strong>Reviewer 1:</strong> Current CD4 was also associated with LDL-C but again seems not adjusted in multivariate analysis.</td>
<td>We have included CD4 count in the multivariate logistic regression model and now association between current ddI use and increased LDL-c levels did not reach statistical significance (OR= 3.44 (CI95%: 0.976-12.12), p=0.055). This is stated in the results section and has been removed from the discussion section.</td>
<td>Methods section: Lines 193-194</td>
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<td><strong>Reviewer 1:</strong> Are any of the patients having concomitant hepatitis C infection, as this is also associated with insulin resistance?</td>
<td>Seven patients presented with hepatitis C virus co-infection but no association with elevated HOMA-IR was found (p=0.193).</td>
<td>Results section. Lines: 211-212 and 245-247.</td>
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<td><strong>Reviewer 1:</strong></td>
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<td><strong>Was lipodystrophy assessed in this study?</strong></td>
<td>There are no standardized tests for assessment of lipodystrophy in children, and this fact could be a significant bias when interpreting results. Finally it was decided not to include lipodystrophy status in the study.</td>
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<td><strong>Abstract, Methods: “adolescents 12 years of age and older” should be stated as “individuals 12 to 20 years old”</strong></td>
<td>Abstract (methods section) has been changed. &quot;We present results from a cross-sectional analysis including individuals 12 to 20 years of age, from a prospective, longitudinal cohort of HIV-infected children, adolescents and young adults in Madrid.</td>
<td>Abstract: Methods. Lines 97-100</td>
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<td><strong>“insuline resistance” in keywords should be “insulin resistance”. Throughout the text, “insulin” often spelt as “insuline”</strong></td>
<td>We appreciate reviewer’s comments and spelling errors have been corrected throughout the manuscript</td>
<td>All text</td>
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<tr>
<td><strong>Results, first paragraph: “adolescents and young adults” preferred to “children”; “female” preferred to “women”</strong></td>
<td>Terms &quot;Children&quot; and &quot;women&quot; were changed as suggested by reviewer 1</td>
<td>Results: Lines 206 and 207</td>
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</table>
**Reviewer 1:** Throughout the text, “presented” should be changed to “presented with”.

| Presented has been changed to "presented with" throughout the text | All text |

**Reviewer 1:** Tables: some decimal points stated as “,“

| Tables have been corrected | Tables |

**Reviewer 1:** Needs some language corrections before being published

| English was reviewed by a native professional corrector (Mr. Martin J. Smyth, B.A) |  |

**Reviewer 2:** The heading doesn’t fully catch the study objectives and findings. It suggests that the whole study was about looking at the association between waist circumference and insulin resistance. They should consider revising the heading. One option would be to have a more declarative heading or just a general heading.

| Title has been changed as suggested by reviewer: "Lipid and glucose alterations in perinatally-acquired HIV-infected adolescents and young adults". | Title |
**Reviewer 2:** Since this analysis is based on Madrid HIV cohort, it would be nice to provide a bit more background of this main cohort. For example authors could add information on whether clinics involved in these were urban or semi-urban and the length of follow-up period.

According to reviewer's suggestions a this paragraph was modified: "We present results from a cross-sectional analysis performed in 2009, within a prospectively followed CoRISpe-Madrid Cohort of Pediatric HIV-infected children and adolescents that started on 2002. All centers were urban clinics".

**Reviewer 2:** The text on inclusion criteria is very short. It is not clear if length of ARV use was one of criteria used for including patients in these analyses. This is important since the length of ARV use is likely to determine if patients develop complications or not.

Inclusion criteria are listed in methods section "Inclusion criteria included: age between 12 and 20 years old, perinatally-acquired HIV infection, follow-up at one of six participating centers and at least one visit during study period with anthropometric measurements and fasting blood samples available."

**Reviewer 2:** It would also be nice for authors to provide any details for exclusion criteria.

We have included a new description of eligible patients and patients who met inclusion criteria in results section:

"During the study period there were 214 patients followed up in..."
<table>
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<th><strong>Reviewer 2:</strong> One important piece of information which is missing is socio-economic status. If this was collected it would be nice to include this in the analyses as this could confound the relationship between insulin resistance with waist circumference</th>
<th>We appreciate this comment from reviewer. Unfortunately, socioeconomic status was not collected in this study. We have included a paragraph about this limitation in discussion section.</th>
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<td><strong>Discussion section:</strong> Lines: 340-341</td>
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<th><strong>Reviewer 2:</strong> One other major aspect of this study is the selection of participants. It would be nice to provide information on whether the selection was random and how this was implemented. This will help readers judge if the findings are generalizable. If this was not randomized, it could be noted in the results section.</th>
<th>According to reviewer's suggestions we have included a paragraph about selection of participants in results section: “During the study period there were 214 patients followed up in CoRISpe-Madrid Cohort of Pediatric HIV-infected children and adolescents. One hundred and four adolescents and young adults who met inclusion criteria were eligible, and five patients refused to participate in the study. Ninety-nine were enrolled...”</th>
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<tr>
<td><strong>Results section:</strong> Lines: 203-207</td>
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the selection did not ensure equal chance to take part in this study, it may suggest that the selection process may have been biased and this will compromise generalizability of your findings

**Reviewer 2:** The classification of abnormal glucose results did not seem to follow WHO guidelines. No mention if oral glucose tolerance test was done for patients with impaired fasting glucose results. It would be nice for authors to use WHO guidelines in proving results of glucose testing to enhance comparability with other studies or provide justification for not using WHO guidelines.

The American Diabetes Association defined impaired fasting glucose with the cutoff of plasma fasting glucose of 100 mg/dl (ADA. Diabetes Care 2015;38(Suppl. 1):S8–S16). This glucose value (100 mg/dl) has been defined as the optimal cut-off in healthy adolescents in our country (García Cuartero B et al The HOMA and QUICKI indexes, and insulin and C-peptide levels in healthy children. Cut-off points to identify metabolic syndrome in healthy children. An Pediatr (Barc). 2007; 66:481–490.) therefore we have use this cutt off in the study. These two references have been included in the manuscript.

As suggested by the reviewer we have included this paragraph in discussion section (Limitations)

| Methods section: Lines 175-176. Discussion section: Lines 342-346 |
"Oral glucose tolerance was not performed in this study. We use the ADA's cutoff for impaired fasting glucose (100 mg/dl), so it should be noted that other organizations define the impaired fasting glucose cutoff at 110 mg/dl. A lower cutoff for IFG could overestimate the prevalence, but only 4 patients were over 100 mg/dl and insulin resistance definition was not influenced by IFG cutoff."

Reviewer 2: In statistics section, it would be nice to describe how selection of variable into multivariable models was done. In the current version, it is not clear how this selection was done, this information will strengthen this section. Authors could also explore interactions between predictor variables in these models and report them if present.

We appreciate reviewer's comments. This paragraph was modified in methods section: " Adjusted analyses: All variables independently associated were included in the multivariate analysis, as well as those variables considered clinically relevant. Multivariate logistic regression model was used to study the association of ddI exposure with increased LDL-c, adjusted for potential confounders including age, sex, Tanner stage, weight, PI and NRTI treatment length and CD4 nadir. Multivariate logistic regression model was used to study the association of abdominal circumference Z score with increased HOMA-IR, adjusted for potential confounders including age, sex, Tanner stage, weight, PI and NRTI treatment length and CD4 nadir. Interactions between predictor variables were evaluated but no significant results were found, and were not included in the model.

Methods section:
Lines: 190-200
**Reviewer 2:** Except for IR, the prevalence of other metabolic abnormalities does not seem to be different from those for the background population. Authors need to highlight this more boldly.

The prevalence of lipid abnormalities in HIV adolescents compared with non-infected is similar, but a higher rate of hypertriglyceridemia was found among HIV patients. This has been highlighted in a paragraph in discussion section: "In the Spanish pediatric population, the reported prevalence of hypercholesterolemia (> 200 mg/dl) is estimated to be 19.2-26.6% and 13-22% for elevated LDL-c (>130 mg/dl) and, in the Madrid area, 7.7% of children presented with triglycerides over 100 mg/dl [22]. We found similar rates of hypercholesterolemia (27.2%) and elevated LDL-c (14.1%), but a higher rate of hypertriglyceridemia (39.8%) in perinatally-acquired HIV-infected adolescents and young adults."

**Reviewer 2:** Good that authors describe other limitations of this study.

We appreciate reviewer’s comments. We are aware that our study has several limitations, and have tried to highlight this in the discussion section.

We have included in discussion section the next paragraph with limitations of the study: "This study has the inherent limitations of a cross-sectional design, and no causal inferences can be made. The
rather small sample size of the study obviously limits our ability to
measure the contribution of particular ARV regimens on the
development of metabolic abnormalities. Socioeconomic status of the
family was not investigated in this study, and this fact should have
some relation with nutrition and health habits and, therefore, with
metabolic profiles. Oral glucose tolerance was not performed in this
study. We use the ADA's cutoff for impaired fasting glucose (100
mg/dl), so it should be noted that other organizations define the
impaired fasting glucose cutoff at 110 mg/dl. A lower cutoff for IFG
could overestimate the prevalence, but only 4 patients were over 100
mg/dl and insulin resistance definition was not influenced by IFG
cutoff. Lastly, the absence of a control group of uninfected children
constitutes a main limitation of this study. In order to overcome this
fact, a referenced-based population has been used for comparison,
and parameters have been adjusted according to age, sex and Tanner
stage. In order to overcome this fact, a referenced-based population
has been used for comparison, and parameters have been adjusted
according to age, sex and Tanner stage".
| Author | There is a mistake in author's order. Dr. MI González-Tomé should be in last place. | Authors |