Author's response to reviews

Title: Effects of ethnicity and CD4 count on glucose metabolism among HIV patients on highly-active antiretroviral therapy (HAART)

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Author's response to reviews: see over
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Dear Editors:

We appreciate the reviewers’ careful reading of our manuscript, and their constructive critiques. We have responded to all of their queries and suggestions, and believe that the manuscript is much improved as a result. Below are our specific responses to each of the reviewers’ comments.

REVIEWER 1

Major

1. The title of this paper and the goal according to the introduction is to assess the effect of ethnicity... This study design only allows for assessment of the relationship between or association of, and as a cross sectional study is not designed to formally test the effect of any variable on another. I strongly suggest amending the title and stated goal of the study to reflect this.

RESPONSE: The reviewer’s comment is very well taken. This is a cross-sectional study, hence it is not possible in a longitudinal manner to assess the “effect” of ethnicity. We have amended the title and stated goal to indicate that this study was designed to assess the relationship of ethnicity and CD4 count on glycemic regulation in HIV patients on stable HAART.

2. The patients selected, by design, had hypertriglyceridemia ...hypertriglyceridemia is clearly associated with insulin resistance .......Indeed, the HOMA was ~3 which is suggestive of insulin resistance in each group. Given this, it is reasonable to view the subjects as a distinct population from a randomly selected cohort of people with HIV, many of whom will not have high triglyceride levels and lack insulin resistance. This is clearly a design limitation and should be noted as such in the discussion. Further, the findings of this study should be considered to be limited only to patients with high triglycerides.

RESPONSE: The reviewer is correct that the study population comprised a group of HIV patients selected for hypertriglyceridemia, hence with many features of Metabolic Syndrome. This was not a random population cohort. This was clearly indicated in “Methods”. We have modified the Discussion section to indicate that in a strict sense the
results would apply to the subset of HIV patients with hypertriglyceridemia / metabolic syndrome. We respectfully decline to note this as a design “limitation” (a term that carries a pejorative connotation, suggesting a design flaw); HIV patients on HAART have a higher risk than the general population for developing diabetes, and this study population was deliberately selected to represent patients at the high end of the risk scale (but without a history of diabetes) as a group who would be enriched in HIV/HAART-specific factors associated with dysglycemia.

3. A major finding of this study was that in the moderate CD group Hispanics had the highest glycemic excursions whereas in the low group, AA had the worst glycemic changes. This is all predicated on the cut-off of 300. While the authors provide a rationale for this cut-off I would like to know if cut-offs of 350 or 200 are do these change the findings?

RESPONSE: With a cut-off of 200, there were very few subjects in the <200/cc category [NHW=8; AA=2; Hispanic=9], hence results of the analysis could not interpreted meaningfully. When we used the 350 cut-off, we obtained results very similar to those presented in the paper using the cutoff of 300/cc.

4. It is hard to know what to make of the findings of an interaction of ethnicity with glycemic excursion. Is it possible that this is a fluke finding or was this a pre-specified goal of the study? If CD4 level was not associated with dysglycemia, the authors should explain why they then sought to determine if this was affected by ethnicity.

RESPONSE: This was not a fluke finding, as our pre-specified hypothesis was that ethnicity and CD4 count (i.e., a stable level of CD4, representing optimal immune reconstitution, on stable HAART) would exert an influence on glycemic regulation in hypertriglyceridemic but otherwise healthy HIV patients. The reviewer is correct that we did not make this sufficiently clear in the originally submitted manuscript – we have clarified this point in the revised paper.

5. It is hard to believe that whole-body glucose concentration is dramatically affected by differences in CD4 glucose uptake. I agree that systemic inflammation could be involved but the hsCRP values were if anything lower in the low CD4 group.

RESPONSE: In regard to the first point: a) the total mass of CD4 cells in humans is very large, even in patients with “low” CD4 counts – for a patient with 100 cells per mm$^3$ or $1 \times 10^5$/ml, the total number of circulating CD4 cells would be in the range of 3-4 x $10^8$ cells, and this is not accounting for the fact that most (perhaps 90-95%) of the CD4 cells in the body are within lymph nodes and other tissues – collectively, therefore, CD4 cells constitute a metabolically active organ of significant size. Moreover, there would be a log-order higher number of total blood CD4 cells in patients with a “high” CD4 count; b) CD4 T cells can be markedly active in regard to glucose uptake and glycolysis; in particular, when they are in an “effector” state (stimulated with antigen) Glut-1 associated glucose uptake and subsequent glycolysis increases markedly, the latter in preference to oxidative metabolism (Michalek et al, J Immunol 2011 Mar 15;186(6):3299-303. doi: 10.4049/jimmunol.1003613). This could be the situation with CD4 cells that respond well (with increased proliferation and expansion) to HAART. Moreover, Glut-1 mediated glucose uptake into CD4 cells is coupled with HIV entry and thus is a positive regulator of HIV infection (Loisel-Meyer et al, PNAS 2012 Feb 14;109(7):2549-54. doi: 10.1073/pnas.1121427109). Hence, it appears reasonable to speculate in the Discussion that variations in CD4 number or degree of active HIV
replication or transport in CD4 cells could produce differences whole body glucose disposal.

In regard to the second point - there is no difference in the CRP value between the groups distinguished by CD4 strata, but CRP (in the context of HIV infection) tends to reflect acute inflammation. It is not a measure of chronic inflammation or immune activation that occurs in HIV infection due to gut microbial transfer. In this regard, sCD14 might be a more reliable marker, but unfortunately we did not make that measurement, so there is little we can say about the relationship of HIV-associated systemic inflammation to glucose levels.

6. As noted above, the discussion should not that the findings of this study cannot be extrapolated to the HIV population as a whole, but only those with high triglycerides or other signs of insulin resistance.

RESPONSE: As indicated in the response (above) to comment #2, we have modified the Discussion section to indicate that the results apply to the subset of HIV patients with hypertriglyceridemia / metabolic syndrome.

Minor

7. As noted by the authors, it is not surprising that A1C was higher in AA as most studies have shown about a 0.2-0.3 point higher A1C for any given glucose in AA than Caucasians and were actually about that expected. Indeed the mean A1c for AA was almost in the prediabetic range. What proportion of each group met A1C criteria for diabetes and/or prediabetes.

RESPONSE: 15 NHW [21.7% of total = 20.3% prediabetic (A1c 5.7-6.4) and 1.4% diabetic (A1c >= 6.5)], 14 AA [45.2% of total = 35.5% prediabetic and 9.7% diabetic], and 16 Hispanic [18.3% of total; 12.6% prediabetic and 5.7% diabetic].

8. As A1c covaried with ethnicity, I believe that the statistical methods employed would tend to favor showing that there was an interaction between ethnicity, A1C and fasting glucose, so I think it is hard to know what to make of this finding (bottom of page 9-10).

RESPONSE: We agree with the reviewer. Since interaction terms are difficult to interpret, and since P for the interaction was slightly > 0.05, we have removed that sentence from the table and results section.

9. The table is too long. I would delete height, weight, hip and leave BMI, adiposity, waist and waist:hip ratio and perhaps fat-free mass. Consider deleting the 30-90 minute time-points in the OGTT in the table to make it more readable, could just such incremental or absolute AUC.

RESPONSE: We thank the reviewer for this suggestion and have modified the table accordingly.

10. The association of PI use with lower fasting glucose is interesting as several studies have suggested that efavirenz increases fasting glucose (Levitt NS. Effect of nonnucleoside reverse transcriptase inhibitor-based antiretroviral therapy on dysglycemia and insulin sensitivity in South African HIV-infected patients, JAIDS). Is it possible to determine if efavirenz was associated with elevated fasting BG in the database?

RESPONSE: In this data set, there was no significant difference in fasting glucose values between those subjects who took efavirenz and those who did not. The mean ± SD for the groups were 94 ± 13.1 and 97.4 ± 18.3, respectively; P = 0.189.
11. Lines 4-6 page 10, are grammatically incorrect
RESPONSE: We have corrected grammar here and throughout the paper.

REVIEWER 2
1. Why only a population of hypertriglyceridemic patients were selected? HyperTG is a multifactorial finding in HIV, frequently associated with the use of PI-based therapies. No clear explanation for this selection bias was provided. The results of the study can be applied only for patients on ART with HyperTG, this must be described in abstract conclusions.
RESPONSE: As noted above in response to Comments #2 and 5 of Reviewer 1, the study population comprised a group of HIV patients selected for hypertriglyceridemia. This was clearly indicated in “Methods”, but we have now also noted this in the Abstract conclusions. We have also modified the Discussion section to indicate that the results would apply to the subset of HIV patients with hypertriglyceridemia / metabolic syndrome. HIV patients on HAART have a higher risk than the general population for developing diabetes, and this study population was deliberately selected to represent patients at the high end of the risk scale (but NOT already meeting criteria for diabetes) as a group who would be enriched in HIV/HAART-specific factors associated with dysglycemia.

2. No mention is made on NRTI backbone combination: Thymidine analogues clearly influence on diabetes risk.
RESPONSE: The number of subjects who were taking thymidine analogues was 60 [19 NHW, 13 AA, 28 Hispanics; 12 with CD4 < 300 and 48 with CD4 ≥ 300]. There was no significant difference in fasting glucose values between those subjects who were taking thymidine analogues and those who were not [P = 0.646]. Thymidine analogue use did not vary by CD4 Strata or Ethnicity [P=0.341 and 0.122, respectively]. We have made a note of this in the text.

3. First-generation PI (indinavir, saquinavir, nelfinavir) were associated with higher glucose metabolism abnormalities than the more recent ones (atazanavir, darunavir). A brief description of what PI were more commonly used must be provided.
RESPONSE: Of the 60 subjects who reported use of PI drugs, 25 (13 NHW, 2 AA, 10 Hispanics) were on first generation agents (indinavir, saquinavir, nelfinavir), and 35 (13 NHW, 5 AA, and 17 Hispanics) were on the more recent agents (atazanavir, darunavir). Analysis showed that there was no difference in the frequency of subjects on the older compared to the newer PI drugs in relation to CD4 Strata or Ethnicity [P=0.534 and 0.061 respectively]. We have made a note of this in the text.

3. Median viral load is 2,41 log; The proportion of undetectable (<50) was similar between groups?
RESPONSE: The proportion of subjects with undetectable viral load was similar among CD4 Strata and among the ethnic groups [P=0.236 and 0.564 respectively]. We have made a note of this in the text.

4. No mention is made to % of patients with HCV/HIV coinfection, which may influence glucose metabolism.
RESPONSE: Fourteen subjects [6.9%] had HIV/HCV co-infection. However, mean fasting glucose was similar between those with and without the co-infection [P=0.204].
5. Lipodystrophy is associated with more profound glucose abnormalities. The proportion of patients with this complication must be provided. 

RESPONSE: We did not systematically assess lipodystrophy in a quantitative manner, as the condition is quite heterogeneous and there is lack of consensus on how to quantify or grade the different forms (lipoatrophic vs. lipohypertrophic vs mixed vs lipomatosis, etc), especially in multiethnic populations such as ours. Since qualitative approaches to defining lipodystrophy are prone to subjective error, we cannot confidently provide this information; however, in a qualitative, subjective manner, less than 10% of the subjects overall had visibly apparent facial or limb lipoatrophy.

6. Table 1 is extraordinarily complex and very difficult to read. I suggest reducing its content, eliminating minor rows (eg 90 min OGTT on some indices, such as HOMA-B, weight and height, hip circumference, etc.), or

RESPONSE: As noted above in response to Comment # 8 of Reviewer 1, we thank the reviewer for this suggestion and have modified the table accordingly.

REVIEWER 3

1. First of all, table 1 is excessively long and difficult to be read. 

RESPONSE: As noted above in response to Comment # 8 of Reviewer 1 and Comment #6 of Reviewer 2, we have modified the table to make it easier to read.

2. The absence of the post-hoc control after the first inter-group comparison limits the possibility of deriving those conclusions provided by authors.

RESPONSE: As suggested by the reviewer, we have updated the statistical analysis and results section with Post-Hoc analysis. For the statistical analysis section, we have added the sentence “When difference in groups were observed, Bonferroni Post Hoc analysis was performed to determine which groups were different from each other.” In the results section, we have mentioned that Bonferroni Post Hoc tests were performed.

3. Moreover, as you performed ANOVA you considered the continuous variables as "normally-distributed", but the majority of variables you presented appeared to be non-normally distributed. In this setting, you needed to use non-parametric test (i.e. Wilcoxon or Kruskal-Wallis tests, using some probability correction [as Bonferroni]) or to transform variables.

RESPONSE: For all the models, normality of the outcomes conditional on the covariates was assessed. We found that for each outcome, the normality assumption held.

4. Another major statistical concern is about CD4 stratification: it has not been specified whether in the ethnicity category CD4 cell count are equally distributed or not.

RESPONSE: As suggested by the reviewer, we have re-checked the number and percentage of participants by ethnic category and CD4 strata. Chi-square analysis and cell frequency/percentage show that the percentages of NHW, AA, and Hispanics are fairly equally distributed in the two CD4 strata [P =0.195].

5. Table 2 provides many concerns. According to the table, the variable "Ethnic category" was analyzed as an ordinal variable, not as a categorical one. This could affect the whole analysis and consequently your conclusions.

RESPONSE: While we agree that misrepresentation of a categorical variable as ordinal
is incorrect, we did treat ethnic category as a “non-ordinal” variable. Within the statistical package used for analysis, the variable was defined as a categorical variable. Non-Hispanic White subjects were considered as the referent group in the model since this ethnic group had the largest sample size and most data in the literature pertain to this ethnic group.

On behalf of myself and my coauthors, I thank you in advance for your consideration and look forward to hearing from you.

Sincerely,

Ranjita Misra