Author’s response to reviews

Title: Insulin Resistance and Adipokines serum levels in a Caucasian Cohort of HIV-positive Patients Undergoing Antiretroviral Therapy: a cross sectional study

Authors:

Victoria Arama (dr.arama@mateibals.ro)
Catalin Tiliscan (catalin_tiliscan@yahoo.com)
Adrian Streinu-Cercel (strega@mb.roknet.ro)
Daniela Ion (danielalion7@yahoo.com)
Raluca Mihailescu (ralsan@gmail.com)
Daniela Munteanu (danielaimana.mn@gmail.com)
Adriana Hristea (adriana_hristea@yahoo.com)
Sorin S Arama (sorinarama@gmail.com)

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Author’s response to reviews: see over
Dear Dr. Ole-Petter Hamnvik and Dr. Nicola Gianotti,

Thank you for your expert review of our manuscript entitled **Insulin Resistance and Adipokines serum levels in a Caucasian Cohort of HIV-positive Patients Undergoing Antiretroviral Therapy: a cross sectional study.** Your comments were very helpful.

Based on your comments we revised our manuscript and we added new results, after a close examination of our study database.

**Dr. Ole-Petter Hamnvik**

Specific comments.

1. Discretionary revisions:
   - I would discourage the use of “complex” antiretroviral therapy. All HIV therapy is complex, and the commonly used terminology is simply “antiretroviral therapy”.

   Please note that, based on all comments, we changed “complex” antiretroviral therapy into combination antiretroviral therapy (cART).

2. Minor essential revisions:
   - I would recommend defining the QUICKI cut-off point in the abstract.
   - Also define the abbreviation IR in the abstract.
   - Please clarify that p-values used are two-tailed.

   Please note that we modified the text based on your comments.

3. The authors state that the effects of ART are acute as they are not associated with duration of therapy. As all participants had taken ART for at least 6 months, I would clarify this statement.

   Please note that we modified the text based on your comment.

4. Page 3: Please change “hypertrigliceridemia” to “hypertriglyceridemia”. Also change “associates with” to “is associated with”.
   - Page 7: Change typographical errors: “observed” rather than “obsedved”, “type 2” rather than “type II”, “regression” rather than “regresion”.
   - Page 8: Change “surogate” to “surrogate”.

   Please note that we modified the text based on your comments.

5. Major compulsory revisions:
   - I would include the incidence of insulin resistance using HOMA-IR also.

   Please note that we modified the text and provided median values and IQR for HOMA-IR.

6. The authors claim to be giving the interquartile range for non-normally
distributed variables in their table. However, they are only supplying a single number in the parentheses. I would recommend changing to median (25th percentile – 75th percentile). For example, 23.8 (21.0 – 30.2).

According to several authors, the interquartile range (IQR) measures statistical dispersion and is equal to the difference between the upper and lower quartiles: IQR=Q3-Q1. IQR is a robust statistic and we preferred this to Q1 and Q3 values.

We provide below a reference:

http://books.google.ro/books?id=vXzWG09_SzAC&pg=PA55&dq=interquartile+range&redir_esc=y#v=onepage&q=interquartile%20range&f=false

7. In Table 1, I would include an initial column with the overall characteristics of the population.

Please note that we modified the table based on your comment.

8. We also need a bit more information about the population in terms of baseline prevalence of metabolic risk factors, such as hyperlipidemia, hypertriglyceridemia and hypertension (all as categorical variables in addition to the continuous variables already presented).

Please note that we modified the table based on your comment.

9. The authors are not including age or BMI in their regression model, but this is a must as both of these are known confounders between adipokines and insulin resistance. Hence, although these variables do not reach significance due to the modest n, including them is essential in order to make appropriate conclusions. Also, the women in the study are younger, could this explain the different findings between the genders?

As we have stated in our paper, we wanted to build statistical linear regression models with the best correlation coefficients, while still having statistical significance. First we tested individual variables, including age and BMI. The variables with univariate association were added to the models. Also, important confounders, such as age or BMI, were added in the additive models. We provide in figure 1 and figure 2 the univariate linear regression models for age and BMI, that lacked statistical significance. Also, we provide a multivariate analysis for male subjects in figure 3, that includes age and BMI, along with LOGAdiponectin and LOGTriglycerides. Although this model reaches statistical significance, the individual variations of independent variables within the model are not statistically significant. We chose to present in our paper only the models with complete statistical significance.

Based on your comment we modified the text and we specified that BMI and and age are included in the linear regression analysis. We do not have a specific reason that may explain the difference of age between genders. To our knowledge, this age difference is not a feature of our Romanian HIV cohort.
Figure 1. Univariate analysis: effect of BMI on QUICKI (dependent variable)

<table>
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<tr>
<th>Model</th>
<th>Variables Entered</th>
<th>Variables Removed</th>
<th>Method</th>
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Model Summary

<table>
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<th>Adjusted R Square</th>
<th>Std. Error of the Estimate</th>
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ANOVA

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<td>.085</td>
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Coefficients

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<th>Standardized Coefficients</th>
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<td>Std. Error</td>
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<tr>
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</table>

Figure 2. Univariate analysis: effect of age on QUICKI (dependent variable)

<table>
<thead>
<tr>
<th>Model</th>
<th>Variables Entered</th>
<th>Variables Removed</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Age</td>
<td>.</td>
<td>Enter</td>
</tr>
</tbody>
</table>

Model Summary

<table>
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<th>R Square</th>
<th>Adjusted R Square</th>
<th>Std. Error of the Estimate</th>
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<td>.013</td>
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ANOVA

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<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
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Coefficients

<table>
<thead>
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<th>Model</th>
<th>Unstandardized Coefficients</th>
<th>Standardized Coefficients</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>Std. Error</td>
</tr>
<tr>
<td>1</td>
<td>(Constant)</td>
<td>.329</td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td>.000</td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td>.000</td>
</tr>
</tbody>
</table>
The authors are suggesting screening for insulin resistance in their conclusion. However, though their data shows a high incidence of insulin resistance, there is no evidence that there is any benefit in screening for insulin resistance per se. I would agree that it is necessary to screen for metabolic complications of ART, such as diabetes mellitus, hyperlipidemia and hypertriglyceridemia, and hypertension, but that is not the focus of this paper. Would recommend removing this recommendation.

Please note that we agree and removed this recommendation.

11. Would expand on the limitations imposed by study design and number of participants.
   - The correlations seen are small to moderate in magnitude, and this should be mentioned in the paper

Please note that we agree and modified the text according to your comment.

**Dr. Nicola Gianotti**

Specific comments.
1. Abstract. “Insulin resistance … is related to antiretroviral therapy.” should be changed to “Insulin resistance … maybe related to antiretroviral therapy.” The association is not so straightforward and many concomitant causes (cART being...
only one of these) likely exist.

Please note that we modified the abstract according to your comment.

2. Abstract and text. “... complex antiretroviral therapy (cART).” should be changed
to “... combination antiretroviral therapy (cART).” which is the usual meaning of
the acronym cART. Furthermore, not all of the antiretroviral regimens are
complex (e.g. single tablet regimens).

Please note that we modified the text according to your comment.

3. Methods. The author should measure insulin resistance also by HOMA-IR (see
below).
Results. The author should report data on insulin resistance also as measured by
HOMA-IR (see below). This does not mean that all of the analyses should be
repeated also with HOMA-IR as outcome, but at least a description of the
population by HOMA-IR values must be reported.

Please note that we modified the text and provided median values and IQR for HOMA-IR.

4. If authors have data on the familiar history for diabetes, these data should be
mentioned, reported and included in the analyses.

Unfortunately we do not have the necessary data for all the patients.

5. Page 6. “…LOGAdiponectin with LOGTriglycerides remained associated with
QUICKI index (R=0.43, p=0.007) in males.” Same R and p-value for both
LOGAdiponectin and LOGTriglycerides?

Please note that this correlation coefficient and p-value are for the statistical model that
includes both LOGAdiponectin and LOGTriglycerides. Individual p-values are provided in
table 3, as shown below.

| Table 3. Multivariate analysis: effect of selected variables (LOGAdiponectin,
<p>| LOGTriglycerides) on QUICKI (dependent variable) in male subjects. |
|-------------------------------|-----------------|-----------------|-----------------|-------|</p>
<table>
<thead>
<tr>
<th>Independent Variables</th>
<th>Unstandardized Coefficients (B)</th>
<th>Standardized Coefficients (beta)</th>
<th>95% Confidence interval for B</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOGAdiponectin b</td>
<td>0.009</td>
<td>0.278</td>
<td>(0.000; 0.001)</td>
<td>0.04</td>
</tr>
<tr>
<td>LOGTriglycerides b</td>
<td>-0.013</td>
<td>-0.261</td>
<td>(-0.026; 0.01)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

LOGAdiponectin - log transformed adiponectin serum levels computed variable for linear regression

LOGTriglycerides - log transformed triglycerides serum levels computed variable for linear regression

6. The authors present results of analyses shifted by gender, but no analysis on the
overall population: as the size of the population is relatively small, also an
analysis of the overall population must be presented: analyzing only subgroups
separately, the numbers become very small, giving the results a very low
Please note that we performed this analysis in our revised text.

7. HbA1C was not measured? If yes, results should be reported.

Please note that we report HbA1c values in our revised text.

8. References 26 and 27 are inadequate: there are a number of studies more relevant than those cited addressing this issue.

Please note that we modified the references according to your comment.

9. QUICKI is one algorithm to measure insulin resistance; however, it is not necessarily the best one and it not widely used: other authors suggest that HOMA-IR should be the preferred one (Wallace and Matthews. Diabet Med. 2002 Jul;19(7):527-34.). Anyway, differently from QUICKI, HOMA-IR has been linked to clinical outcomes in HIV-infected patients in many studies (a few examples: Gianotti et al.HIV Med. 2011 Feb;12(2):109-17; Bigoloni et al.AIDS. 2012 Sep 10;26(14):1837-40; Hessol et al.J Acquir Immune Defic Syndr. 2012 Oct 15. [Epub ahead of print; Freitas et al.BMC Infect Dis. 2012 Aug 6;12(1):180; Al-Fadhli et al.Med Princ Pract. 2012 Jun 21; Veloso et al.Cytokine. 2012 May;58(2):253-60; etc). Furthermore, the absence of at least descriptive data on HOMA-IR prevents from comparisons between the results of the present study and those from previous studies.

Please note that we modified the text and provided median values and IQR for HOMA-IR.

10. Table 1 and table 2. Data on fasting glucose and HbA1C are missing. At least fasting glucose values must be presented.

Please note that we modified the table and provided the necessary data.

Thank you for your kindness and expertise in reviewing our manuscript.