Author's response to reviews

Title: Abdominal obesity and low physical activity are associated with insulin resistance in overweight adolescents: A cross-sectional study

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Author's response to reviews: see over
We appreciate the comments of the evaluators because those help us to improve the article. Here we answer their questions:

**Evaluator N° 1: Silvia Regina Saldiva**

**General comments:**

The manuscript addresses an important public health condition: the possible mechanisms for the development of insulin resistance amongst adolescents. Authors conclude that visceral adiposity, as inferred by measures of abdominal circumference, and sedentarism are key players for the development of insulin resistance. The topic is not new, since there are many studies on this topic, but authors stated in line 97 that few studies focused on adolescents that already developed insulin resistance as consequence of their weight status. This point has no references and should be expanded by the authors, in order to make clear what is the new information provided by their study.
Response to the reviewer: We accept the suggestion from the evaluator; in fact, the affirmation that we made was not based on any reference, the confusion is due to a wrong drafting. What we wanted to explain was that the study is focus in adolescents that developed IR as a consequence of their overweight.

For clarity the text is modified at line 99 to 104: “Many studies have characterized the metabolic and inflammatory alterations, as well as the lifestyles, of overweight adolescents (25-33). This study examined adolescents who have already developed IR because of their weight status and assessed the associations between IR and the anthropometric, metabolic, inflammatory, food consumption, and physical activity (PA) variables in overweight adolescents.”

Major compulsory

a) Dietary habits were obtained using a 24-hours recall. In order to overcome the limitations of this approach when evaluating a chronic condition, in line 172 authors reported that a second questionnaire was distributed amongst a random subsample to calculate intra-individual variation. Neither the number of individuals nor the results of this approach were presented. Without this piece of information, it is not possible to assess the reliability of the data of food intake, mainly in the context of the limitations of 24-hours recall to evaluated feeding habits. The usual instrument to evaluate food habits is the Food Frequency Questionnaire, as proposed by Willet in Nutritional Epidemiology, 3rd edition, chapter 5, and page 74. Authors should expand the text in order to make clear the limitations of the employed approach.

Response to the reviewer. As suggested by the evaluator, dietary habits can be better evaluated by a Food Frequency Questionnaire. However, our interest in this
study was to evaluate specifically, the calories and nutrients intake of one person during one day, this is achieved with a 24-hours recall. From the viewpoint of public health, the really interest is the nutrients average intake by each person; this is call as well like habitual intake or usual intake. The nutrients habitual intake was estimated by the methodology proposed by Nusser and collaborators (Nusser SM, Carriquiry AL, Dodd KW, Fuller WA. A Semiparametric Transformation Approach to Estimating Usual Daily Intake Distributions. J Am Stat Assoc. 1996;91(436):1440-49). We used the software PC-SIDE version1.0 from 2004, this was developed by the statistical department, Iowa State University, Ames IA, EEUU, the software esteem the distribution of habitual intake on the population and the habitual intake of one nutrient for each individual; this has good statistical properties and it is known as the Best Linear Unbiased Predictor. (MPLI) for the habitual intake of one person. For this is necessary to have a 24-hours recall for all population, distributed in the seven days a week, and a second recall for a minimum of 10% of the study population; with this information the software adjust the own intra-individual variation of food consumption. This method has been used in some national studies like the National Survey of Colombia, 2005(ENSIN 2005) (Instituto Colombiano de Bienestar Familiar, Profamilia, Instituto Nacional De Salud, Escuela de Nutrición y Dietética Universidad de Antioquia, Organización Panamericana de la Salud: Encuesta nacional de la situación nutricional en Colombia, Bogotá; 2006).

The text is modified at line **176**: “Food consumption. To obey with the goal to evaluate calories and nutrients intake for one person by day, a 24-hours recall was randomly distributed during different weekdays. A second questionnaire was also
distributed amongst a random subsample constituted by 20% of the study population (24 adolescents) to calculate intra-individual variation (50,51). The dietary intake evaluation program (EVINDI v4) was used (52-55). Nutrient reports were generated using the PC version of the Software for Intake Distribution Estimation (SIDE) program, Iowa State University, version 1.0, June 2004”

b) Odds ratios were computed by univariate approaches and the multivariate model was based on linear regression. Was the dependent variable in the multivariate models a dichotomous variable? If so, the beta coefficient does not represent the probability of occurrence of IR after adjustment for the explanatory parameters. Perhaps authors should consider a polychotomous multivariate logistic model. Another point to be considered is the occurrence of nonlinear associations between IR and the independent variables.

Response to the reviewer: Its assessment is correct and we appreciate your observation because we expressed inappropriately the calculations about multiple linear regression model and the OR. The dependent variable in the regression model was the HOMA value; this is a reason variable (numeric) continuous, which is why we used a multiple linear regression model. This model fulfilled the assumptions of linearity, normality, constant variance, independence and collinearity. For this model we did not use the classification of HOMA (dichotomous variable that classifies IR). That is why we did not use a logistic regression. In fact, the Odds ratios were computed by univariate approaches.

On the text, line 204 to 210 was modified the drafting about statistical analysis: “Chi-square tests were used to calculate the association between
categorical variables. Pearson’s and Spearman’s coefficient were used to assess the correlations between variables. The probability of IR occurrence was calculated by Odds ratios (ORs). Through multiple linear regression model was estimated the average value of HOMA (dependent variables) according to the presence of independent variables (hsCRP, WC and PA).

On the line 265 were better drafting the model results: “In the initial exploratory data analysis, the variables that show association with HOMA were tested in a multiple linear regression model, in the process were introduce the variables one by one and the variable that was not significant was discarded. Multiple linear regression model explained 43.3% of the variance in the associations between HOMA and hsCRP, WC, and PA. However, only WC and PA significantly explained HOMA. For every 1 cm increase in WC, the HOMA index increased by 0.082, and for every MET increase in PA, the HOMA index decreased by 0.026. This model fulfilled the assumptions of linearity, normality, constant variance, independence and collinearity (Table 5).”

The table 5 in the line 485 was adjusted with information about the assumptions of the model.

Table 5. Multiple linear regression between HOMA index and IR-explicatory variables

<table>
<thead>
<tr>
<th>Variables</th>
<th>B</th>
<th>CIs</th>
<th>p</th>
<th>VIF</th>
</tr>
</thead>
<tbody>
<tr>
<td>hsCRP (mg/L)</td>
<td>0.01</td>
<td>-0.06-0.08</td>
<td>0.707</td>
<td>1.007</td>
</tr>
<tr>
<td>METs/day</td>
<td>-0.03</td>
<td>-0.05 - -0.01</td>
<td>0.013</td>
<td>1.004</td>
</tr>
</tbody>
</table>
Finally, authors should present a sensitivity analysis, to define how robust is their estimate of risk for developing IR to model specifications, considering the possibility of collinearity amongst the set of explanatory variables.

Response to the reviewer. With the linear regression model we could explain the behavior of HOMA from some independent variables. However, this regression equation could not be used to estimate HOMA in a consultation and to define IR, because the two associated variables just explain the 43.3% of the phenomenon, and the range of the proposed target in this study was not to establish a predictive model but a behavioral explanation of HOMA. The authors propose that the two variables associated with HOMA can be considered in the anamnesis assessment of young during the process of health care to detect those that require confirmatory laboratory tests of IR. For the above reasons, we did not make the sensitivity analysis that was suggested by the evaluator.

d) Authors should explain with more detail the criteria to choose 3 variables from the entire set of measured parameters
Response to the reviewer. In the initial exploratory data analysis those variables that showed association with HOMA were proven by the model. The variables were introduced one by one, and those that were not significant were discarded. We find that from the variables composing the final model (hsCRP, WC y PA), only two: WC y PA were significant. The model explained the HOMA behavior in a 43.3%.

The text was complemented in line 265: “In the initial exploratory data analysis, the variables that show association with HOMA were tested in a multiple linear regression model, in the process were introduce the variables one by one and the variable that was not significant was discarded. Multiple linear regression model explained 43.3% of the variance in the associations between HOMA and hsCRP, WC, and PA. However, only WC and PA significantly explained HOMA. For every 1 cm increase in WC, the HOMA index increased by 0.082, and for every MET increase in PA, the HOMA index decreased by 0.026. This model fulfilled the assumptions of linearity, normality, constant variance, independence and collinearity (Table 5).”

Furthermore, the text was complemented in line 375 to 384 with the following explanation in the discussion: “The results of the present study suggest that a WC >p90 and sedentary behaviour are associated with IR, lipid profile alterations, higher inflammatory status, and CVD risk amongst overweight adolescents. This two variables associated with HOMA, could be considered in the anamnesis assessment of adolescents with overweight during the process of attention in health to detect those that required confirmatory laboratory tests of IR. Thus a proper screening including BMI and WC evaluations by health personnel might
enable the early detection of a feature that eventually promotes the development of chronic diseases like diabetes and CVD, with effects not only on the individual but on the health economy with the completion of paraclinical only young people who are at risk”.

**Minor points**

In line 91, authors state that health personnel do not currently use WC in everyday practice. Is this assertion valid to Medellin, to the entire Colombia or is a global habit. A clarification, or a reference, should be included to justify the assertion.

**Response to the reviewer.** The observation is relevant.

We clarify the text on the line 91: “*However, the health institutions in Colombia do not account instruments to measure the WC and the health personnel does not register this information at the clinic history, that is why the health system just measures weight and height to classify overweight by body mass index (BMI)*”.
Evaluator N° 2: Loreana Silveira

Discretionary Revisions (which are recommendations for improvement but which the author can choose to ignore)

- The characterization of the sample and the care of the author in detailing the information must be highlighted. I would suggest the presentation of results comparing the prevalence of overweight and/or IR by this variables, such as socioeconomic strata.

Response to the reviewer. This study aimed to explore the associations between anthropometric, metabolic, inflammatory, food consumption and physical activity variables with presence of IR in adolescents with overweight. Therefore, we did not make the comparison between the presence of overweight and IR with the socioeconomic strata variable, although we report in table 1 the frequency by strata. The socioeconomic strata variable did not permit to do a discrimination by study’s groups because the majority of adolescents belonged to medium strata, followed by low strata and just 12.5% belonged to high strata, leaving a very low n in each group that hinder the statistical analysis.

- In the methodology, could also be included the Lohman’s equation used to calculate the %BF.

Response to the reviewer. we accept the suggestion.

We adjust the text at line 137: “Total body fat percentage (%BF) was calculated using TFFs and SFFs according to the Lohman equation: \[
\text{Σ folds } >35\text{mm } \%BF= 0.783 \times \Sigma \text{TFFs,SFFs}+1 \text{ (men)} \text{ and } \%BF= 0.546 \times \Sigma \text{TFFs,SFFs}+9.7 \text{ (women)}; \quad \text{Σ folds } <35\text{mm } \%BF= 1.21(\Sigma \text{TFFs,SFFs})-0.008(\Sigma \text{TFFs,SFFs})^2+1\text{ (men)} \text{ and } \%BF= 1.33(\Sigma \text{TFFs,SFFs})-0.013(\Sigma \text{TFFs,SFFs})^2+2.5 \text{ (black women: 2.0 and white women:}
3.0). Obesity was classified as %BF >25% for boys and >32% for girls, NW was classified as 12-25% for boys and 15-32% for girls (39).

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

- In the session of limitations of the study, the authors affirm the inexistence of a validated methodology of PA measurement. I would say that we do have some good instruments of quantification of PA, such as the accelerometer. The method used in the study was clearly a limitation, as highlighted by the authors, however it limitation can be explained by studies which has already compared both methodologies. (Anderson CB1, Hagströmer M, and Yngve A. Validation of the PDPAR as an adolescent diary: effect of accelerometer cut points. Med Sci Sports Exerc. 2005 Jul;37(7):1224-30.)

Response to the reviewer. We accept the suggestion.

We adjust the text at line 369: “On the other hand, PA was assessed in young people using the method 3DPAR, because the study did not have a better instrument like accelerometer. However, Cheryl B (74), in their validation study of the method PDPAR, demonstrated moderate correlations between this questionnaire and the accelerometer MTI, similar or higher to the correlations found with another self-report measures”.

- One of the most important results of the present study was the non-association of calorie intake between the groups, as expected, but was the PA the major determinant in the IR. It should be well explored in the discussion, emphasizing the importance of PA even the intermittent daily exercise and its benefits in health and RI.
Response to the reviewer. We believe that the approach given to the association between PA and RI in the discussion is sufficient; as this was done using actual studies support this finding, meta-analysis of 2014 recommendations for the prevention of overweight and metabolic alterations was obtained in teens. We discussed about the benefits of PA and changes in lifestyle. We suggest recommendations that can be useful at educational institutions with the purpose of increase the time of PA, due to the clear conclusion is that PA to low increased risk of IR in overweight adolescents.

- Regard to the previous consideration, I would suggest the inclusion of a new multiple linear regression table having the PA or MET/day as the dependent variable and compare with HOMA index and IR-explicative variables. It would help in the discussion of PA and prevention of IR.

Response to the reviewer. In light of the purpose of the study, we pretended to investigate which biochemical and lifestyle variables can explain the HOMA behavior as a dependent variable, in order to find an explanation to the presence of RI in overweight adolescents. This is consistent with the hypothesis proposed at the beginning of the research: “The RI in overweight adolescents is associated with abdominal obesity (high WC), low PA levels and high caloric intake”; therefore considered to pose a regression model where the independent variable is HOMA would change the purpose of the study.

- The substantial consideration about the manuscript is the possibility of identification of IR in adolescents using an accessible methodology. In other words, if there is the possibility of assess PA with a questionnaire instead of accelerometer, or identify RI by HOMA instead of oral tolerance glucose test, it means an easy
measurement of risk factors in children and adolescents. I suggest the authors to emphasize the importance of their findings in epidemiological prevention of metabolic syndrome risk factors.

**Response to the reviewer:** The regression equation found in this study could not being used as a tool to detect RI in a population screening because the PA and WC variables explain the phenomenon only in 43.3% and scope of the proposed objective in this study was not to establish a predictive model but a HOMA explanatory behavior. What the multiple linear regression model could provide to the clinic practice is that this two variables: WC and PA are strongly associated with HOMA and these can be considered in the assessment of adolescents during the consultation to detect those requiring confirmatory laboratory tests of IR, which not only allow early detection of feature that eventually involve the development of chronic diseases such as diabetes and CVD, but also improves the health economy, with the completion of paraclinical only young people who are at risk.

**Based on the above, we adjust the text at line 375:** “The results of the present study suggest that a WC >p90 and sedentary behaviour are associated with IR, lipid profile alterations, higher inflammatory status, and CVD risk amongst overweight adolescents. This two variables associated with HOMA, could be considered in the assessment of adolescents with overweight during the process of attention in health to detect those that required confirmatory laboratory tests of IR. Thus a proper screening including BMI and WC evaluations by health personnel might enable the early detection of a feature that eventually promotes the development of chronic diseases like diabetes and CVD, with effects not only on
the individual but on the health economy with the completion of paraclinical only young people who are at risk”

Major Compulsory Revisions (which the author must respond to before a decision on publication can be reached)

• In table 1, the sex percentage of NW group should be corrected to 47.5.

Response to the reviewer: Table 1 was corrected. Male: 52.5% female: 47.5%.