Author's response to reviews

Title: Insulin Resistance and its Association with the Components of Metabolic Syndrome among Obese Children and Adolescents.

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Author's response to reviews: see over
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Title of the paper: **Insulin Resistance and its Association with the Components of Metabolic Syndrome among Obese Children and Adolescents**

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On behalf of my co-authors, please find enclosed the manuscript mentioned above, submitted for consideration to BMC Public Health. The manuscript has been carefully revised and includes changes according with the suggested recommendations emitted by the referees. All changes can be identified because they are highlighted in yellow or green. Please note that table 3 contains new data – it is in fact an altogether new table. In the following paragraphs we provide answers – in blue – to each one of the referees’ comments.

**Answer to reviewer: D Thivel**

1. I am not a native English speaker and then clearly understand the difficulties of writing in such a language, and I would suggest the authors to make their paper review for a better understanding.
   
   A. The first version of the manuscript was sent to a professional copy editor whose native language is English (American Journal Experts Editorial. Certificate key 5FCF-D593-0BAD-76F4-6D78). This new version has been carefully checked and edited so as to make it more comprehensible.

2. In their background, the authors state that many papers have exposed the problem with metabolic syndrome definitions in pediatric population. Many papers have been effectively published about these MS definition difficulties; it would be great to cite at least one of them.
A. We now have included some references which point at the difficulties involved in defining MS. (references numbers 13, 14).

3. In the result section, data are presented in the text without any precision regarding the standard deviations, which would be great to correct. P values are also missing in the result section in the text ("girls exhibited smaller concentration of fasting glucose, total CHO and LDL (p...)."

A. The result section now includes the missing standard deviations, percentages and P-values.

4. The authors propose a great discussion with appropriate references however their conclusion (abstract) is too strong. Indeed, they state that "an increased degree of IR increases the incidence of metabolic disorders and the risk of metabolic syndrome in children and adolescents". Regarding their data analyses it appears to direct to formulate the conclusion in such a way, it would be better to conclude in an association between the IR degree and the MS parameters.

A. We thank the reviewer for this thoughtful observation. The conclusion (abstract) was modified so as to ascertain that a strong association is observed between degrees of IR and MS parameters.

Answer to reviewer: Sophie Hawkesworth

Major compulsory revisions:

1. I have some concerns about the appropriateness of the analysis conducted in this paper. Both the independent variable, which in this case is insulin resistance (IR), and the dependent variable (the metabolic syndrome: MS) contain a measure of fasting glucose and are therefore interrelated. I would question whether it is thus correct to investigate the risk of developing MS depending on the IR status of the obese children studied? Instead, the paper could be restructured to look only at the association between IR and the other components of the metabolic syndrome in these children (removing fasting glucose) if this was felt to remain a valid research question.

A. HOMA-IR values are indeed obtained from the relationship between glucose and insulin concentrations. However, one must bear in mind that, though being related, HOMA-IR values and fasting glucose levels maintain independence from each other as variables. Thus, when insulin sensitivity diminishes in skeletal muscle and adipose tissue (thus impairing glucose uptake), there is an increase in the concentrations of insulin as a mechanism to keep fasting glucose within normal limits. Therefore, IR can exist alongside "normal" values of fasting glucose. For this reason we consider necessary to explore the association between fasting glucose and HOMA-IR values.

2. If this new approach was taken I would suggest that Table 4 and Figure 1 were no longer necessary and that the analysis presented in Table 3 could be improved. For example, what is the rationale for categorizing the HOMA-IR variable into percentiles? It may be more informative to conduct a linear regression analysis
investigating the relationship between (in turn) HOMA-IR and waist circumference, HDL-C, Triglycerides, Systolic and Diastolic blood pressure. These analyses could be conducted both as simple regression and adjusted for appropriate confounders such as age, sex and BMI.

A. Following the reviewer’s suggestions, we conducted linear regression analyses to investigate the relationship between HOMA-IR and the different components of MS. These analyses, however, yielded no relevant information. For this reason, we kept the organization of HOMA-IR variables into percentiles in order to ascertain the cut-off point at which the components of MS become cardiometabolic risk factors. The current table 3 shows the OR, 95% confidence intervals and p-values for each of the components of MS. All of these were obtained through logistic regression analysis adjusted for age and gender. We decided to keep table 4, which shows the association between IR and risk of presenting MS. The rationale for this is that IR has been suggested as a common pathway to developing MS among adults, and this model seems valid for children too.

3. The paper would also be improved by greater clarity of the study design. For example, how were the schools selected for the first stage of the study and how were the 466 children selected for the second stage of the study from the 1475 eligible to participate?

A. In the methods section we now state that all public primary schools of Campeche City were included in the study. Likewise, we have now mention that, from the total population of obese children, 600 children were randomly selected and invited to participate. The 466 children who are reported in the study are those from whom we were able to obtain complete blood samples.

3.1 The discussion would also benefit from acknowledgement of the limitations of this type of cross-sectional analysis.

A. In the last paragraph of the discussion (page 12, rows 5-6) we now mention the limitations of this type of cross-sectional analysis.

Minor essential revisions:
1. There are some problems with the language and grammar used in the paper which will require careful editing. For example: ‘overweightness’ should be replaced by ‘overweight’ throughout.

A. In the current version of the manuscript “overweightness” has been replaced by “overweight” throughout. By the way, the term “overweightness” was suggested by a professional translator (American Journal Experts Editorial Certificate key 5FCF-D593-0BAD-76F4-6D78) to whom the manuscript was sent before submitting it to BMC Public Health

2. Page 4, line 7. You state that obesity increased by 1.1% annually amongst school children from 1999-2006. Is this based on just the two surveys mentioned (1999 and 2006)? If so, it is not possible to conclude if this was a 1.1% annual increase or if the rate varied between years. The 1999 survey should also be referenced here.
A. In the current version of the manuscript we now provide the information requested by the reviewer. Both the 1999 and the 2006 surveys are now referenced (ref 1, 2). According with the differences between two surveys, the annual increment as an average was 1.1 percent points.

3. Page 5, line 7. You state that the prevalence of MS in children is unknown. Do you mean in Mexico? If so, this should be clarified.
   A. We do not mean in Mexico, at least not only. The prevalence of MS in children is unknown not only in Mexico but in many other places. The current version of the article provides information about the worldwide prevalence of MS among both the general population of school-age children and overweight school children (page 5, rows 3-10). We also point at the main reasons why there is no agreement regarding the concept of MS.

4. Did trained research personnel conduct the measurements of blood pressure and waist circumference? How many different personnel took these measurements and is there any data on the variation in their measurements?
   A. In the methods section we now state that all the measurements (height, waist circumference and blood pressure) were made by four nurses trained for this purpose. No data is available on the variation in their measurements.

5. Please explain the rationale for the 3.4 cut-off for IR in the text on page 7. Does this reflect to the distribution of HOMA in this population?
   A. The cut-off point that defined IR was 3.4. This value corresponds to the 90th percentile of HOMA-IR in a healthy population, and has been suggested as a cut-off point to define IR among children and adolescents (reference 24 of the manuscript). On another note, this same value corresponds to the 50th percentile of our population with obese children.

6. Page 7, line 10. I am unclear as to what ‘measures of central tendency and dispersion’ refers to. Please clarify.
   A. In the methods section it is now stated that we obtained the mean and standard deviation.

7. Page 7, line 12. Please remove the ‘y’ before ‘#75th’
   A. We have removed the “y” before ‘#75th’.

8. Page 8. Please be consistent when you report the differences between boys and girls in the components of the MS studied. For example, please include in brackets the percentages for hypercholesterolemia and LDL as well as the p-values.
   A. The results section now shows in parentheses the percentages for hypercholesterolemia and LDL as well as the p-values for both girls and boys.

9. Please use the specific p-values throughout the text rather than cut-offs of
P<0.001 etc.
   A. We have now used the specific P-values throughout.

10. Page 9, line 14. Please explain what you mean by the fact that a higher pubertal stage could explain the fact that girls have higher insulin levels and lower glucose than boys.
   A. In page 10 we explain that, because girls enter puberty earlier than boys, at equal ages, their more “advanced” pubertal development leads girls to have higher hormonal concentrations (growth hormone, sexual steroids) which, in turn, favour weight-gain and a greater likelihood of developing "physiological" IR.

11. Page 11, line 16. You state that the high prevalence of ‘metabolically healthy, but obese individuals’ suggests a protective genetic feature but your data may just reveal that other metabolic disorders develop later than obesity, which is a more likely explanation and one that should be emphasized.
   A. In the current version of the manuscript we stress the large number of children already metabolically affected (87%), but we also point at the protective role that genetics could play herein. Nevertheless, we also point out a limitation of the study: the impossibility of knowing whether currently healthy children will remain so in the future or will go on to develop disorders in the future.

12. Table 1. The ‘&’ sign after HOMA-IR should be superscript.
   A. This has been corrected.

13. Table 2. The reference you give for the HOMA-IR cut-off does not contain this information and is different from the one provided previously in the text. Please amend.
   A. This has been amended; the correct reference is number 24.

Answer to reviewer: Douglas Curran-Everett

Minor Essential Revisions
1. Percentages and P values throughout manuscript. Is 0.1% all that important? Please round percentages to integers. P values can be rounded to 0.01 unless they are less than 0.01 in which case rounding to the nearest 0.001 is sufficient.
   A. Both within the text and the tables, percentages and P-values have been rounded to integers.

2. Statistical analysis, p 7. By variance analysis do you mean analysis of variance? Logistic regression analysis is sufficient. The adjective multivariate is unnecessary.
   A. The section on statistical analysis was modified following the reviewer’s suggestions. Now it reads “logistic regression analysis.”

3. Tables, presentation of SD. The +/- with SD is inappropriate: a SD is a single positive number. Means and SD can be better presented as mean (SD). For
example, in Table 1, age for all subjects is better presented as 11.3 (0.8). The format can be defined in the explanation to the table.

A. We have followed this suggestion. The format is defined in the explanation to the table (at the bottom).

4. Table 3. It seems to me the values in Table 3 can be rounded to the nearest integer.

A. The table has been modified. As it is now, table 3 shows the odds ratio according to the different categories of IR.

5. Figure 1. My copy of Figure 1 had a blank space in the middle of the broken line between 0 and 3+ components of metabolic syndrome.

A. Due to a mistake of ours, figure 1 did not show the following text: \( \Delta \text{HOMA-IR} = 2.9 \) (103.6\%) which corresponds to the difference between the first and the last column. We will make sure to send the complete figure this time.

We thank the referees for all their thoughtful observations and comments.

We very much look forward to hearing from you.

Yours sincerely,

Samuel Flores-Huerta MD