Author's response to reviews

**Title:** Insulin resistance (HOMA-IR) cut-off values and the metabolic syndrome in a general adult population: effect of gender and age. EPIRCE cross-sectional study.

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**Author's response to reviews:** see over
Dear Editor,

Thank you for considering our manuscript “Insulin resistance (HOMA-IR) cut-off values and the metabolic syndrome in a general adult population: effect of gender and age. EPIRCE cross-sectional study“.

Enclosed please find our revised manuscript, (Ref. No.: MS: 3133073926855834) addressing the reviewer’s critiques. We have attempted to address all of the comments and suggestions provided by the reviewers. We believe that the manuscript has been greatly strengthened by these suggestions.

We hope that these revisions will meet with your approval, and we look forward to your review of our revised manuscript,

Kind regards,

Pilar Gayoso

We have changed the order of Tables accordance with the reviewer. In the review main document: Table 1 is the previous Table 4; Table 2 is the previous Table 1; Table 3 is the previous Table 2; and finally Table 4 is the previous Table 3.

The Figure 1 legend “Figure 1. Performance of HOMA-IR levels for classification of cardio metabolic risk in non-diabetic population. Influence of age and gender in the area under curve (AUC) distribution, ROC regression models.” was change by “Figure 1. Performance of HOMA-IR levels for classification of cardio metabolic risk in non-diabetic population. Influence of age and gender in the area under the ROC curve (AUC), ROC regression models.”
Responses to reviewers.

Reviewer #1: Fumihiko Kamezaki

#1. For a better understanding of this study by the majority of readers of the Journal, the authors should revise the Abstract section in more detail. For example, the authors do not show the definition of MetS and the methodology of ROC curve analysis. In addition, the reviewer thinks that the authors have to clearly show the study results and concisely conclude this study. Also, the authors should avoid suddenly using abbreviations such HOMA-IR, IR, ROC, and MetS.

Response: The abbreviations IR and Mets were removed and we spell out HOMA – IR as homeostasis model assessment. We think that “ROC regression” is a very common expression in scientific papers and to include “receiver operating characteristics regression” no add more useful information to readers.

The Abstract section has been replaced with the text:

“Background. Insulin resistance has been associated with metabolic and hemodynamic alterations and higher cardio metabolic risk. There is a great variability in the threshold homeostasis model assessment (HOMA-IR) levels to define insulin resistance. To describe the influence of age and gender in the estimation of homeostasis model assessment of insulin resistance (HOMA-IR) optimal cut-off values to identify subjects with higher cardio metabolic risk in a general adult population.

Methods. It included 2459 adults (range 20–92 years, 58.4% women) in a random Spanish population sample. As an accurate indicator of cardio metabolic risk, Metabolic Syndrome, both by the International Diabetes Federation criteria and by the Adult Treatment Panel III criteria, were used. The effect of age was analyzed in individuals with and without diabetes mellitus separately. ROC regression methodology was used to evaluate the effect of age on HOMA-IR performance in classified cardio metabolic risk.

Results. In Spanish population the threshold value of HOMA-IR drops from 3.46 using 90th percentile criteria to 2.05 take into account of Metabolic Syndrome components. In non-diabetic women, but no in men, we found a significant non-linear effect of age on the accuracy of HOMA-IR. In non-diabetic men, the cut-off values were 1.85. All values are between 70th-75th percentiles of HOMA-IR levels in adult Spanish population.

Conclusions. The consideration of the cardio metabolic risk to establish the cut-off points of HOMA-IR, to define insulin resistance instead of using a percentile of the population distribution, would increase its clinical utility in identifying those patients in whom the presence of multiple metabolic risk factors imparts an increased metabolic and cardiovascular risk. The threshold levels must be modified by age in non-diabetic women.”

#2. More methodological details should be provided regarding the measurement for fasting insulin. In addition, the reviewer thinks that researches about the HOMA-IR should at least exclude subjects with the use of antidiabetic drugs in order to diminish the possibility of inaccurate evaluations of HOMA-IR. Antidiabetic drugs and high levels of fasting glucose may influence the study results in diabetic men and women.

Response: We add the kit used to measure fasting insulin. In Methods section, (P 5, Ln 15-16) Specific laboratory determinations, the text “Fasting insulin levels were measured using a radioimmunoassay (RIA) method.” was replaced with “Fasting insulin levels were measured using a radioimmunoassay (RIA) method using the ….. kit (marca).”

In the Methods section, Setting second paragraph, page 4, it has been describe the sample’s characteristics. The study population include people with diabetes (247, 10%) and without diabetes (2212, 90%). Diabetes was defined as fasting plasma glucose levels ≥126 mg/dL (ADA
criteria) and/or the current use of antidiabetic drugs in individuals with diabetes diagnostic confirmed.

All the analyses of data were performed separately by diabetes status. We have added the following text in Methods section, Statistical Analyses (P6, Ln 19-21): “Since it is well established that HOMA-IR values behave differently according to gender and diabetes status, the analyses were performed separately in men and in women and in diabetic and non-diabetic individuals.”

#3. Why don't the authors use the updated criteria of MetS (Reference 8) by six major world organizations including the International Diabetes Federation? Abdominal (central) obesity is not the sole criterion for the diagnosis.

Response: We agree with the reviewer that the abdominal obesity is not the sole criterion for the MetS diagnosis, but as Alberti et al says the waist measure would continue to be a useful preliminary screening tool.

In order to compare our results with other populations and to provide more useful information to clinical practitioners, we employed the criteria of MetS that were more frequently used in clinical setting, at least in Spain, and in previous epidemiological studies: International Diabetes Federation and National Cholesterol Education Program Adult Treatment Panel III (ATP III).

#4. The authors should show the proportions of post-menopausal women, and examine the impacts of menopausal changes on the HOMA-IR levels.

Response: It would certainly be of great interest this analysis suggested by the reviewer, however no contrasted information was available about menopause in all women included in the sample. Therefore this variable was not included in the present study.

Minor Essential Revisions:

#1. The authors should not use abbreviations including CVD, DM2, and CKD because such words are not repeatedly use in the text (Background, 10th paragraph and Methods, 6th paragraph).

Response: Abbreviations were removed in Background, 10th paragraph and Methods, 6th paragraph.

#2. Is the sentence (Methods, 14th to 15th paragraph) “The average age was 49.4±16.2 years (range 19-92 years)” correct? The authors describe the EPIRCE study included Spanish persons aged 20 years and older (Methods, 4th paragraph).

Response: There are a mistake in this sentence. The text: “The average age was 49.4±16.2 years (range 19-92 years)” has been replaced by “The average age was 49.4±16.2 years (range 20-92 years)”.

#3. The method of measuring waist circumference should be provided (Methods, 21th paragraph). In women, waist circumference at the umbilical level is thought to be several centimeters longer than that measured at the midway between the lowest rib and the iliac crest.

Response: The United States (US) National Institutes of Health (NIH) protocol provided in the NIH Practical guide to obesity (NHLBI Obesity Education Initiative, 2000) and the protocol used in the US National Health and Nutrition Examination Survey (NHANES) III indicate that the waist circumference measurement should be made at the top of the iliac crest.

We have added the following text in Methods section, 21th paragraph: “To measure the waist
circumference all researchers followed these instructions: Locate the top of the hip bone (iliac crest) and take the measurement just above this bony landmark, just where one finger can fit between the iliac crest and the lowest rib. Ensure that the tape measure is positioned horizontally, parallel to the floor. Measuring at a level just above the iliac crest, and positioning the tape horizontally, irrespective of whether the umbilicus is above or below the tape, provides the correct waist circumference measurement and should correspond to the maximal abdominal diameter. Ensure that the patient is standing erect and has relaxed the abdominal muscles. Measurement must be taken at the end of normal expiration.

#4. Are the continuous data normally distributed? If the data are not normally distributed, they are often presented median and quartiles, for instance.

Response: The continuous data were normally distributed. Kolmogorov-Smirnoff test for the hypotosis of normal distribution was used.

#5. The authors have to revise the sentence “Women had a significantly higher waist circumference component than men (43.6% vs. 29.8%, P<0.0001) (Results, 6th to 7th paragraph)”. There are differences in cutoff levels of waist circumference between the International Diabetes Federation criteria and the Adult Treatment Panel III criteria.

Response: We agree with reviewer; there is a mistake in the text and the data relative to waist circumference component (IDF criteria) had no been included. The sentence “Women had a significantly higher waist circumference component than men” has been replaced with “In contrast, women with positive MetS component of larger waist circumference were significantly higher than men (43.6% vs. 29.8%, ATPIII criteria, 52.7% vs. 35.1%, IDF criteria, P<0.001)”

#6. The authors should provide high-resolution figures (Figure 1 and 2 are unclear for the readers).

Response: The Figure 1 and Figure 2 in JPEG format has been replaced by TIFF format to provide high-resolution.

#7. The authors should show the numbers of each age group in Table 2 and 3.

Response: In non-diabetic women, we found a significant non-linear effect of age on the accuracy of HOMA-IR in identifying cardio metabolic risk, both ATP III and IDF criteria of MetS. Therefore to illustrated the performance of HOMA-IR in this group, the AUC (Table 2, now Table 3) and HOMA-IR cut-off values (Table 3, now Table 4) were estimated, just as an example, for three ages: 30, 50 and 70 years.
Reviewer #2: Qiuhua Shen

Gayoso-Diz et al investigated the effects of age and gender on the cut-off values of HOMA-IR in identifying subjects with metabolic syndrome among general Spanish adults, using cross-sectional data from the EPIRCE study (n = 2459). This is an important topic and the research question was well defined. The methods used in the study were appropriate and well described. The findings from this study will provide useful information to the study pool of this important research topic. However, the manuscript could be further improved with the following minor essential revisions.

1. Background: little background information was provided. Instead, the purpose of the study was presented. Please provide brief background information about HOMA-IR cut-off values.

Response: The sentence “There are great variability in the threshold homeostasis model assessment (HOMA-IR) levels” was added to Background section.

2. Background: Ln1, please change “Background” to “Background.”; change “describes” to “describe”; Ln2, please change “identifying” to “identify”.

Response: The text: “Background To describes the influence of age and gender in the estimation of HOMA-IR optimal cut-off values to identifying…” has been replaced by “Background To describe the influence of age and gender in the estimation of HOMA-IR optimal cut-off values to identify…”

3. Methods: the title and the purpose of the study indicated that the effects of age and gender on HOMA-IR cut-off values would be examined in this study; however, no information about gender was provided. Please add this piece of the information.

Response: The text “It included 2459 adults in a random Spanish population sample” has been replaced with “It included 2459 adults (range 20–92 years, 1436, 58.4% women) in a random Spanish population sample “

4. Methods: Ln1-3, “The effect of age on the accuracy of HOMA-IR was analyzed…”, please specify the accuracy of HOMA-IR in determining what (subjects with metabolic syndrome or subjects with insulin resistance??).

Response: That text has been replaced with “It included 2459 adults in a random Spanish population sample. As an accurate indicator of cardio metabolic risk, Metabolic Syndrome, both by the International Diabetes Federation criteria and by the Adult Treatment Panel III criteria, were used. The effect of age was analyzed in individuals with and without diabetes mellitus separately. ROC regression methodology was used to evaluate the effect of age on HOMA-IR performance in classified cardio metabolic risk. “

5. Results: Ln2, please change “…to 2.05 take into account MetS components.” to “…to 2.05 taking into account of MetS components.”
Response: The text: “...to 2.05 take into account MetS components.” has been replaced by “...to 2.05 taking into account of MetS components”.

BACKGROUND

6.P1, Ln1, the authors used “type 2 diabetes”; while in P2, Ln4, “diabetes mellitus type 2 (DM2)” was used. Please keep it consistent throughout the text.

Response: Expression “diabetes mellitus type 2 (DM2)” was used throughout the text.

7.P3, Ln7-8, the sentence of “However, in no case take into account the ability of classification proposed cutoff points are in terms of clinically relevant outcomes” is confusing. Please re-write and clarify.

Response: The text: “However, in no case take into account the ability of classification proposed cutoff points are in terms of clinically relevant outcomes” has been replaced by “However, no take into account the ability of proposed cutoff points to identify risk of clinically relevant outcomes”.

8.P4, please combine this paragraph with P3 by “In addition, in these studies the results ....”

Response: This change has been made.

9.P5, Ln2-3, “On the other hand, the prevalence cardio metabolic diseases such as ...”, please add “of” between “prevalence” and “cardio metabolic diseases”.

Response: This change has been made.

10.P6, Ln4, please delete “serum” and change “identifying” to “identify”.

Response: This change has been made.

METHODS

Setting

11.P1, Ln1, please change the first sentence to “The present study was a secondary analysis of data from a survey of the Spanish general adult population (EPIRCE)”.

Response: This change has been made.

12.P1, Ln5, please change “the adult Spanish population” to “the Spanish adult population”.

Response: This change has been made.

13.P2, Ln4, please change “sex” to “gender”. Anthropometric and clinical measurements

Response: This change has been made.
14. P2, Ln3-5, it was mentioned here that individuals were classified into normal weight, overweight, or obese according to their BMI; however, no data related to this information were reported in the Results section.

Response: Considering that this information is not used in the present paper, the sentence “Following standard criteria, individuals were classified as normal weight (<25 kg/m$^2$), overweight (25–30 kg/m$^2$), or obese (>30 kg/m$^2$).” was removed.

Definition of metabolic syndrome

15. P1, Ln4, please add “cm” after “# 80”.

Response: This change has been made.

Statistical Analyses

16. P1, Ln 3-4, please provide a brief justification of the use of the Mann-Whitney U-test and Kruskall-Wallis test.

Response: In P1, Ln 3-4 after “The Mann-Whitney U-test and Kruskall-Wallis test were employed for comparison of quantitative variables” we added the sentence “Although normally distribution of quantitative variables were verified, non parametric test were used because they are less likely than the parametric test to spuriously indicate significance due to the presence of outliers, they are more robust.”

17. P2, Ln3, please add “is” between the words of “it” and “well” in the sentence of “Since it well established that HOMA-IR values behave differently according to .... in men and in women.”

Response: This change has been made.

RESULTS

18. P1, a brief description of the significant results presented in Table 1 should be provided after the first sentence “Table 1 summarizes anthropometric, clinical, and biochemical characteristics of the study population.”.

Response: We add the sentence: “In the overall data set, the MetS prevalence was 15% for MetS$^{IDF}$ (19.2% in men vs. 12.1% in women, P<0.0001) and 12.7% for MetS$^{ATPIII}$ (14.9% in men vs. 11.1% in women, P=0.006).” after the first sentence.

19. P1, L1-2, first sentence “Table 1 summarizes anthropometric, clinical, and biochemical characteristics of the study population.”, please change “population” to “sample”.

Response: This change has been made (now Table1 is Table 2)

20. P2, Ln 1-3, please move this paragraph to P1 and in the front of the sentence of “In non-diabetic individuals, but not in diabetic individuals, we found significant differences by gender in components of MetS (data no shown)”. This will help the flow of presenting the data (discussing the data presented in Table 1 and followed by data not shown in Table 1).

Response: This change has been made (data presented in Table 2, previously Table 1).
21. P1, Ln4-7, the sentences were somewhat confusing. Please re-write to convey the information that the percentage of men with positive MetS components of higher triglycerides, blood pressure, and glycemia was significantly higher than in women. In contrast, women with positive MetS components of larger waist circumference were significantly higher than men.

Response: This change has been made.

AUC values of HOMA-IR by gender and diabetes status

22. Since the data related to the effect of age on HOMA-IR was also presented under this subheading, it is suggested that “age” should be included in the subheading too (i.e., AUC values of HOMA-IR by age, gender, and diabetes status).

Response: This change has been made.

23. P1, Ln1, please add a brief description of Table 2 and Figure 1 before the first sentence of “Regardless of diabetes status…” (i.e., The results of the effects of age and gender on the accuracy of HOMA-IR (AUC values) among non-diabetic and diabetic individuals were presented in Table 2. Figure 1 shows the estimated AUC values by age, with the corresponding 95% point wise bootstrap confidence band in non-diabetic men and women.)

Response: We added the sentence: “The results of the effects of age and gender on the accuracy of HOMA-IR (AUC values) among non-diabetic and diabetic individuals were presented in Table 2. Meanwhile, Figure 1 shows the estimated AUC values by age, with the corresponding 95% point wise bootstrap confidence band in non-diabetic men and women.”

24. P2, please combine this paragraph with P1.

Response: This change has been made.

25. P2, Ln4-6, please delete the sentence of “Figure 1 shows the estimated AUC values by age, with the corresponding 95% point wise bootstrap confidence bands.” after a brief description of Figure 1 is added in P1.

Response: This change has been made.

Cut-off values of HOMA-IR

26. P2, please combine this paragraph with P1.

Response: In the first paragraph the sentence ““Table 3 shows gender distribution of HOMA-IR cut-off values, with their corresponding sensitivity and specificity” was replaced with “Table 3 shows gender distribution of HOMA-IR cut-off values, with their corresponding sensitivity and specificity both in diabetic and non-diabetic populations. In non-diabetic individuals we found significantly differences by gender”.

27. P4, Ln1-2, the sentence of “All values are between 70th and 75th percentile of HOMA-IR values in the Spanish adult population.” may be deleted as this was also discussed in the
Discussion section.

Response: This change has been made.

DISCUSSION

28. P3, please correct the following editorial errors:

a. Ln3, please add a period at the end of the sentence of “...for MetSATPIII in women”.

Response: This change has been made.

b. Ln4, please delete the extra “.” before “.065” and please add “)” after “0.70”. c. Ln7, please change “we no found...” to “we did not find...”.

Response: This change has been made.

29. P4, Ln2-3, please provide references to the statement of “Recent studies reported marked gender differences with regard to degrees of IR and body composition.”

Response: The references have been added and the sentence has been replaced with “Recent studies reported marked gender differences with regard to degrees of IR and body composition [24, 26].”

30. P5, Ln1, please spell out “IR” as “Insulin resistance” as it is at the beginning of the sentence.

Response: This change has been made.

31. P5, Ln4, it is late to introduce Table 4 here in the Discussion section. The authors could have mentioned this table in the Background section in P3, Ln 5, after introducing previous population based studies “...in different geographic areas [15-22]”.

Response: In Background section in P3, Ln 5, after “in different geographic areas [15-22]”. this text was added “Table 1 shows the cut-off values, as can be seen...”. The Tables has been renumbered: Table 1 to Table 2; Table 2 to table 3; Table 3 to Table 4.

32. P5, Ln7-10, the same statement of “The consideration of the attendant risk of cardiovascular and metabolic diseases to establish this cut-off point would increase its clinical utility in identifying those patients in whom the presence of multiple metabolic risk factors imparts an increased metabolic and cardiovascular risk.” appeared three times in the manuscript, including the Abstract, Discussion, and Conclusions. Please re-write/paraphrase to avoid repeating the exact statements three times.

Response:

33. P5, Ln11, was the threshold value of HOMA-IR using 90th percentile criteria (3.46) found in the present study or was it reported in the previous studies? This value was not reported in the Results section. If it was from the present study, please add to the Results section. If not, please provide reference.
Response: The threshold value of HOMA-IR was reported in the previous study (Gayoso-Diz P, Otero-González A, Rodríguez-Alvarez MX, Gude F, Cadarso-Suarez C, García F, De Francisco A. IR index (HOMA-IR) levels in a general adult population: Curves percentile by gender and age. The EPIRCE study. Diabetes Res Clin Pract 2011, 94:146-155). The reference [24] was added to the sentence: “In Spanish population the threshold value drops from 3.46 using the 90th percentile criteria [24]…”

CONCLUSION

34. P3, Ln 1, please change “sex” to “gender”.

Response: This change has been made.

REFERENCES

35. The format of the references list did not follow the BMC Endocrine Disorders reference style. Please refer to the examples of the BMC Endocrine Disorders reference style provided in the Instructions for Authors and reformat the references list to ensure the style is followed precisely. For example, all named authors (up to the first 30) should be listed before adding “et al.”; the title of a journal article and volume should be bold; Journal names should be abbreviated according to Index Medicus/MEDLINE and italicized.

Response: The format of the references has been revised following the BMC Endocrine Disorders reference style. All named authors have been listed in each reference.

36. Reference #13, please spell out “IR” as “Insulin resistance” in the title.

Response: This change has been made.

37. Table 1 Please add the numbers of subjects who had metabolic syndrome among women and men with or without diabetes, using ATP III and IDF criteria. For example, 11.1% (n = ...).

Response: This change has been made (now Table 2 is the previous Table 1).

38. Please add “HDL, high density lipoprotein” to the footnote of Table 1. Table 2

Response: This change has been made. The footnote of Table 2 (previous table 1) has been replaced with “Data are presented as mean estándar deviation or percentages (n). BMI, body mass index; HDL-Cholesterol, high density lipoprotein-Cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; ATP III, Third Adult Treatment Panel, IDF, International Diabetes Federation.”

39. Please add footnotes for the abbreviations of HOMA-IR, ATP III, MetS, IDF in Table 2.

Response: This change has been made (now Table 3 is the previous Table 2). The footnote of Table 3 has been replaced with “AUC (95% CI), area under the ROC curve (95% Confidence Interval). *ROC regression models incorporating age as covariate. **The AUC was estimated for three ages (30, 50, and 70 years) to illustrate the performance of HOMA-IR. HOMA-IR, homeostasis model assessment of IR; ATP III, Third Adult Treatment Panel; IDF, International Diabetes Federation; MetS, Metabolic Syndrome.”
Table 3

40. Please add footnotes for the abbreviations of HOMA-IR, ATPIII, MetS, IDF in Table 3.

Response: This change has been made. (now Table 4 is the previous Table 3). The footnote of Table 4 has been replaced with "In non-diabetic females HOMA-IR cut-off values are estimated for 30, 50, and 70 years of age, because there is a non linear effect of age on test performance to classify IDF-defined MetS (P value < 0.001) and ATP III-defined MetS (P value = 0.012). HOMA-IR, homeostasis model assessment of IR; ATPIII, Third Adult Treatment Panel; IDF, International Diabetes Federation; MetS, Metabolic Syndrome."

Table 4

41. Please provide the reference numbers for all the references cited in the table.

Response: This change has been made and the reference numbers have been added in the Table 1 (previous Table 4).

42. Cited study of “Taniguchi, 1992” in Table 4 was not found in the reference list. Did the authors intend to refer to Reference #22 (Nakai et al 2002)? If so, please correct the citation. If not, please provide the reference for this study.

Response: Indeed there was an error; the correct reference was Nakai et al, 2002. This change has been made.

Figure 1 and 2

43. The resolution of the two figures was relatively low and the labels for the figures were hard to read.

Response: The Figures 1 and 2 in format jpg have been replaced with Figures 1 and 2 in tiff format.