Dear Mr. Wang,

Thank you for giving us the opportunity to submit a revised draft of our manuscript titled *„Association between cardiorespiratory fitness level and insulin resistance in adolescents with various obesity categories“* to *World Journal of Diabetes*.

We appreciate the time and effort that you and the reviewers have dedicated to providing your valuable feedback on our manuscript. We are grateful to the reviewers for their insightful comments on our paper. We have been able to incorporate changes to reflect most of the suggestions provided by the reviewers. We have highlighted the changes within the manuscript. Here is a point-by-point response to the reviewers' comments and concerns.

<u>Comments from Reviewer 1:</u>

- *Comment 1*: *In the METHOD section, the authors mentioned "according to the terciles of CRF into groups with poor, intermediate or good CRF," but no specific classification criteria exist. There is also a lack of corresponding discussion in the DISCUSSION section, such as how to explain the differences in the CRF intermediate group in Figure 3.*

Response: Thank you for raising the question regarding the classification critera for CRF level. The problem of classification objectively exists, as CRF can be measured, or estimated, using a variety of tests and protocols, and CRF normative measures that are test and protocol-specific are available almost exclusively for healthy children. Obese children and adolescents usually have lower overall physical abilities and lower CRF adjusted to body mass when compared to their normal-weight peers. To our knowledge, the only published reference values for CRF in children and adolescents with obesity were obtained by using Åstrand-Rhyming submaximal bicycle test in 705 Swedish children (Johansson L, Brissman M, Morinder G, Westerståhl M, Marcus C. Reference values and secular trends for cardiorespiratory fitness in children and adolescents with obesity. Acta Paediatr. 2020 Aug;109(8):1665-1671.). However, our study commenced before the aforementioned reference values were published, and the protocol of the study, including the way of CRF classification, was already pre-agreed. Besides, we used submaximal treadmill test, and gaps in knowledge about the interchangeability of results when using different heart rate based tests and protocols still exist. Therefore, we calssified

participants into three categories, according to terciles of CRF achieved in this particular study (the group with the worst third of VO2max values was assigned poor CRF, the group with middle third of VO2max values was assigned intermediate CRF, and the group with best third of VO2max values was assigned good CRF). Although we entirely agree that the classification of CRF according to criteria applicable for obese children and adolescents would be a better choice, due to the previously mentioned reasons, we were unable to do so. Finally, we believe that the applied method of classification allowed us to compare HOMA-IR among the groups of adolescents that differed by CRF level within this study, which was our primary intention.

With respect to the differences in the CRF intermediate group in Figure 3, we didn't discuss it because the differences in the mean HOMA-IR were not statistically significant. However, we agree that this should be mentioned, so we adjusted the text in RESULTS accordingly (page 10, lines 268, 269) .

• Comment 2: *There is a sex difference in adolescent obesity, as mentioned in the DISCUSSION section, "The proportion of participants with severe obesity was higher in adolescent boys than in adolescent girls, which is in line with literature data. " In the description of gender in the first part of the results, the author mentioned "with no statistically significant difference between the sexes"; however, in Table 1, it is shown that in different obesity categories, the gender difference is statistically significant. Secondly, according to Figure 1, it can be seen that female participants are significantly more than males. How should the author explain this difference and eliminate the influence in the study?*

We apologize for the statement in the description of the first part of the results, where we mentioned "with no statistically significant difference between the sexes". We agree that it was not clear enough that this statement refers exclusively to the fact that the mean age of girls and boys was not statistically different. Therefore, we have removed that statement from the text (page 8, lines 225, 226). Assertions "The proportion of participants with severe obesity was higher in adolescent boys than in adolescent girls, which is in line with literature data " and the data from Figure 1 showing that in different obesity categories, the gender difference is statistically significant, are in agreement with each other.

Secondly, in accordance with Figure 1, more girls than boys were included in the study. This can be partly explained by the fact that girls enter puberty earlier than boys, and one of the

inclusion criteria for this study was the presence of puberty. Also, some girls were referred due to irregular periods, hirsutism and skin problems within the framework of polycystic ovary syndrome, which is frequently associated with obesity. Last but not least, dissatisfaction with one's own appearance due to obesity, according to literature, seems to be more pronounced in girls and women. Regarding the elimination of the gender influence on the study results, as stated in the *Statistical analysis* paragraph of MATERIALS AND METHODS, we hope that with the adjustment of all analyses for age and sex, this potential problem was successfully resolved.

• Comment 3: *The aim of this research is "To investigate the association between CRF and insulin resistance in obese adolescents, with special emphasis on severely obese adolescents." However, this study focuses on obese adolescents. Whether it is necessary to set up normal controls is expected to be further explained by the author.*

This is a valid and important question, which we also asked ourselves before the beginning of this study. Normal controls, in case of our study, would be adolescents with normal body weight. According to our knowledge, insulin resistance in Caucasian adolescents is tightly associated with obesity. Authors of a pooled study which included cross-sectional data from three projects, found no significant differences in cardiometabolic risk score and HOMA-IR between fit and unfit normal-weight children, suggesting that the effect of CRF in childhood occurs only in the presence of the obesity phenotype (Nyström, C.D., Henriksson, P., Martínez-Vizcaíno, V., Medrano, M., Cadenas-Sanchez, C., Arias-Palencia, N.M., Löf, M., Ruiz, J.R., Labayen, I., Sánchez-López, M. i Ortega, F.B. (2017). Does cardiorespiratory fitness attenuate the adverse effects of severe/morbid obesity on cardiometabolic risk and insulin resistance in children? A pooled analysis. Diabetes Care, 40(11), 1580-1587). Therefore, we believe that the inclusion of normal weight controls would not be beneficial for our study.

• Comment 4: *The authors classified class II and III obesity as severe obesity, and in a subsequent Two-way ANOVA, only mildly obese and severely obese were analyzed. Why not choose the obesity category (class I/II/III) for analysis?*

Thank you for pointing this out. In literature, different classifications of childhood obesity exist. The one that includes three obesity classes (class I, II and III) is perhaps the most detailed one, and is especially suitable for analysis of prevalence and trends in obesity among children. With

this classification, we wanted to highlight the real extent of obesity in our sample. However, in most studies, CDC definition of severe obesity (BMI ≥ 120% of the 95th percentile or ≥35 kg/m^2) is used. This makes it easier to standardize efforts to better characterize the unique features and risk factors associated with severe obesity, and to evaluate novel approaches for the management of youth with severe obesity. In line with this, we presented the results in a way that adolescents were classified as either mildly or severely obese.

• Comment 5: *It is suggested that relevant results of Two-way ANOVA (Two-way ANOVA with Bonferroni correction) should be presented in tabular form.*

We appreciate this insightful suggestion, and revised the manuscript accordingly (Table 3 and Table 4 are added and mentioned in text page 10, lines 266 and 275).

Comments from Reviewer 2:

Comments: However, there is room for improvement in certain aspects of the paper. For instance, further discussion and comparison of similarities and differences between related studies could be included, as well as elaboration on specific interventions to improve CRF in adolescents with varying degrees of obesity and a comparison of different methods of assessing insulin resistance.
Additionally, it is recommended that the language be polished to enhance clarity and accuracy of expression.

Thank you for encouraging us to further discuss and compare the similarities and differences between related studies. In accordance with your suggestion, we revised the manuscript (page 11, lines 307-309; page 13, lines 343-345; page 13, lines 364, 365; page 14, line 374).

Although we completely agree that the discussion on specific interventions to improve CRF in adolescents with varying degrees of obesity is an extreemly important topic, our study was focused primarily on the association between CRF and insulin resistance in obese adolescents. Therefore, it seems slightly out of scope to discuss on specific interventions for improvement of CRF in detail. However, recognizing the importance of your suggestion, we added a general

recommendation on exercise modalities which are considered to improve cardiometabolic risk in obese adolescents at the end of CONCLUSION (page 15, lines 400,401).

According to your suggestion, a comparison of different methods of assessing insulin resistance wass included in DISCUSSION section (page 11, lines 294-304).

Additional clarifications

In addition to the above comments, the language has been polished to enhance clarity and accuracy of expression.

We look forward to hearing from you in due time regarding our submission and to respond to any further questions and comments you may have.

Sincerely,

Associate Professor Maja Cigrovski Berković, on behalf of Authors