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Editor

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Dear Editor,

We are submitting the revised version of our review article entitled "**Nonalcoholic steatohepatitis and insulin resistance in children**". We thank the reviewer for the comments, which were very helpful for us in improving the manuscript. Our point-by-point responses to the comments are outlined below. We hope that our manuscript is now suitable for publication in *World Journal of Diabetes*. Thank you for considering our manuscript and we look forward to hearing from you.

Yours faithfully,

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Point-by-point responses to the reviewer

Comments to the Author

In the present submission by Arata et al. (Nonalcoholic steatohepatitis and insulin resistance in children), the authors review in short, non-alcoholic steatohepatitis and insulin resistance in children. In the world, the increase in the prevalence of obesity is a serious problem. Associated with this increment, there is a reduction of life expectancy of up to 7 years (Petersen et al., Ann Intern Med 2003; 138:1138-45.). When considering children, it is estimated that 170 million of them (under 18) globally are overweight or obese, affecting more than 20% of all children in many countries (Swinbum et al., Lancet 2011; 378:804-14). In consequence studies focuses in children are always welcome, especially considering the development of strategies to prevent or delay the pathology.

Response

In order to further empahsize the importance of pediatric non-alcoholic steatohepatitis, we added 2 references in Etiology as follows:

"In Japan, 10% of the general population is estimated to have NAFLD, and 1% to have NASH. In adults with obesity and type 2 diabetes insipidus, the rates are higher[3]. A life-table analysis showed a reduction of life expectancy of up to 7 years in adults with obesity[4]. In children, the prevalence of NAFLD/NASH is estimated to be as high as 2.6%-9.6% in the United States and Asian countries, despite significant differences in race and ethnicity[5-7]. Insulin resistance is often accompanied by NAFLD/NASH, and plays a pivotal role in its pathophysiology[8,9]. The prevalence of insulin resistance in obese children foreshadows a worrisome trend for type 2 diabetes. It is estimated that 170 million children under 18 years worldwide are overweight or obese, which is more than 20% of all children in many countries [10]. According to the SERCH for Diabetes in Youth study, more than 20,000 individuals below 20 years of age had type 2 diabetes[11]." (page 3, lines 17-page 4, line 3.)

However in my opinion, several points are not clearly explained.

a) **In general is not easy to distinguish when the authors are explaining the situation in children and when in adults.**

Response

We focused on insulin resistance in children with nonalcoholic steatohepatitis in this manuscript. To make this point clear, we added the following sentences or phrases to the manuscript, as follows.

"The pathological characteristics of NASH are significantly different between children and adults. Nonalcoholic fatty liver disease (NAFLD)/NASH is accompanied by insulin resistance, which plays a pivotal role in its pathophysiology in both children and adults. In NASH, a "two-hit" model involving triglyceride accumulation (first hit) and liver damage (second hit) has been accepted. Insulin resistance was found to correlate with changes in fat levels; however, it did not correlate with fibrosis or NAFLD activity score in children." (in Abstract, page 1, lines 6-11)

"There are no specific symptoms associated with NAFLD and NASH in children. However, there is strong fatigability. Furthermore, obesity, sleep apnea, hypertension, hyperinsulinemia, and acanthosis nigricans are often observed." (in Clinical diagnosis, page 4, lines 8)

"Diagnosis of NAFLD and NASH by conventional blood biochemical examination is difficult. Liver biopsy is required for a definitive diagnosis of NAFLD. For diagnosis, children should be screened for the presence of HBs antigens, HCV" (in Clinical diagnosis, page 4, lines15).

b) **The authors claim that "In children, non-alcoholic fatty liver disease (NAFLD)/NASH is accompanied by insulin resistance". "Significant correlations between insulin resistance**

and NAFLD activity score (NAS)….were found in 177 children with NAFLD/NASH". Is this privative or unique of NAFLD/NASH in children? I do not think so. Adipose tissue insulin resistance is present in the majority of patients with NAFLD, whether patients are obese or not (Buglianesi et al., Diabetologia 2005; 48:634-42). This insulin resistance at the adipose tissue level is critical since there is a close relationship between adipose tissue and liver metabolism in humans because adipocytes supply more than two-third of fatty acids used for hepatic triglyceride synthesis. Fatty acids derived from adipose tissue account for the majority (60%) of hepatic triglyceride accumulation in NAFLD (Donnelly et al., J Clin Invest 2005; 115:1343-51). This is not a unique feature of NAFLD in children. As the authors suggest, "insulin resistance if often accompanied by NAFLD/NASH".

Response

We agree with the reviewer's comments. In accordance with the comments, we changed the following sentences to refer to not only children but to both children and adults, and added the references pointed out by the reviewer, in the Insulin resistance in children with NASH section, as follows.

"Significant correlations between insulin resistance and NAFLD activity scores (NAS), which were calculated by summing the scores for steatosis, lobular inflammation, and ballooning degeneration, were found in 177 children with NAFLD/NASH[30]. Adipose tissue insulin resistance is also present in the majority of adults with NAFLD, whether the patients are obese or not [31]. Reports in the literature on insulin resistance in pediatric NAFLD/NASH are summarized in Table 2[32-40]. These reports demonstrated that insulin resistance is associated with fatty changes using MRI (magnetic resonance imaging) and ultrasound[32,40]. However, insulin resistance was not associated with fibrosis or NAFLD scores (NAS)[32-40].

c) **In my opinion, the Interpretation of the relationship between insulin resistance and fibrosis is obscure. The authors mention that "insulin resistance was found to correlate with changes in fat levels; however, it did not correlate with fibrosis or NASH scores". This assertion refers to adult, to children or both of them? On the other hand, as a "representative" photograph of liver sections of NAFLD/NASH patients the authors**

show a pediatric type with severe fibrosis. If insulin resistance did not correlate with fibrosis and severe fibrosis is "representative" of pediatric type that means that insulin resistance is not "representative" of NAFLD/NASH in children.

Response

That is a major point of this manuscript, and further long-term research on biopsy-proven NASHpatients from the time they are children to when they are adults is required to make any conclusions. Insulin resistance was found to correlate with changes in fat levels; however, it did not correlate with fibrosis or NAS in children. However, insulin resistance correlated with fibrosis without NAS in adults.Therefore, to clarify this point, we made the following changes to the manuscript.

"Insulin resistance was found to correlate with changes in fat levels; however, it did not correlate with fibrosis or NAFLD activity score in children. Therefore, insulin resistance may be important in the first hit. Because there is obvious familial clustering in NASH, genetic predisposition as well as environmental factors including diet might be the second hit of NAFLD/NASH." (page 1, lines 11-13)

"Therefore, these findings suggest that insulin resistance is important for the first hit in the two-hit model of NASH. In adults, insulin resistance did not correlate with NAS but correlated with fibrosis[41,42]. NASH in children is mainly characterized by fatty changes and fibrosis in the portal area (type 2 NASH), which is different to the characteristics of NASH in adults. Therefore, larger scale follow-up studies are required to understand the progression of NASH from children to adults." (page 8, lines 11-14)

d) Recent metabolic studies in patients with NAFLD suggest that liver fibrosis correlates closely with severe adipose tissue insulin resistance (Lomonaco et al., Hepatology 2012; 55(5):1389-97).

Response

In accordance with the reviewer's comment, we added the following additional references regarding fibrosis in NASH/NAFLD in adults.

"In adults insulin resistance did not correlate with NAS but correlated with fibrosis [41,42]." (page 8, lines 10-11).