

## ANSWERS TO REVIEWERS

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Apparently, the original CLA we sent last time did not reach you. We upload again the scanned document with the handwritten signatures.

2. Answer to reviewers: Please provide point to point answer to all reviewers' comments. The comments as follow: The authors of this minireview provided a brief introduction to NAFLD hallmarks, the five groups of animal models available for studying NAFLD and the potential role of metabolomics in the study of experimental NAFLD. The subject of this manuscript is of value, but there are a few of defects need to be modified.

2.1. Should the author describe the current status of NAFLD diagnosis briefly.

We added this paragraph in the first revision of the manuscript. Please, let us know if this was not enough or if it is OK. It is already described in [METABOLOMICS IN NAFLD RODENT MODELS](#) section. I copy the text from the Review:

### METABOLOMICS IN NAFLD RODENT MODELS

Currently, liver function is routinely controlled by blood analysis in which clinicians test for transaminases, albumin, platelets, bilirubin and clotting factors. Patients presenting abnormal levels of these parameters, especially transaminases, and whose medical history reveals risk factors for diabetes, obesity or metabolic syndrome, undergoes a non-invasive imaging method, mainly ultrasonography, to confirm the presence of steatosis and fibrosis in the liver. If the result is positive, the NAFLD fibrosis score and FIB-4 index scores can be applied. Depending on the score, patients are classified as at low, medium or high risk of fibrosis. The goal of these imaging methods is to detect whether fibrosis is present, due to the different follow-up required in patients with fibrosis. An invasive imaging method, biopsy, is performed on those with a high risk of fibrosis or with an unclear diagnosis under non-invasive imaging methods [8, 82-84]. Nowadays, biopsy remains the gold-standard for diagnosis of hepatic steatosis, NASH and fibrosis, as histology confirms tissue damage [7, 8]. Biopsy has a relatively high incidence of false negatives, however, since the fragment finally analyzed only represents about 1/50.000 of the organ and analysis may vary between

pathologists [7]. Moreover, non-invasive imaging methods also present disadvantages. Steatosis can only be detected at over 30% and these methods cannot determine whether NASH is present [85, 86]. We are still far from achieving the main objective: NAFLD prevention and a rapid diagnosis. New non-invasive diagnostic methods are needed, and one alternative could be use of metabolomics in the search for new biomarkers.

2.2. INTRODUCTION section: Non-alcoholic fatty liver disease (NAFLD) .....Knowing that NAFLD reduces in 4 years life expectancy and leads to the appearance of different comorbidities It is suggested that the author mark or exemplify the main comorbidities.

We also added the comorbidities in the first revision. Please, let us know if it is OK or if we need to add anything else. We copy from the Review and we highlight them in blue in the text:

## INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) prevalence has increased drastically in the last decades, affecting up to 25% of the world's population [1]. The rise of disorders such as obesity and type 2 diabetes mellitus, as well as changes in lifestyle and diet composition, have led to a worldwide increase in the incidence of NAFLD [2-5]. Given that NAFLD reduces life expectancy by four years and triggers the appearance of different comorbidities **such as cardiovascular disease, kidney damage or osteoporosis** [3-5], it seems vital for specialists to establish accurate and precise guidelines or strategies to address the disease [6].

2.3. Environmental factors section: Related to environmental factors, it is crucial to highlight dietary habits, reduced physical activity, and even socio-economic aspects associated with the type of diet consumed. This sentence needs to be rewritten more clearly. The language expression of the three factors, dietary habits, reduced physical activity, and even socio-economic aspects, don't seem to match. Reduced physical activity should be changed to: physical activity? Or describe the relationship between the specific changes of the three factors and NAFLD.

The original text was this one:

### *Environmental factors*

Related to environmental factors, it is crucial to highlight dietary habits, reduced physical activity, and even socio-economic aspects associated with the type of diet consumed. The rising number of calories ingested, and consumption of high-sugar and high-fat diets not only increases the risk of developing NAFLD but also of conditions such as obesity and type 2 diabetes mellitus <sup>4,8,12</sup>. It has been shown that lifestyle interventions in diet and physical activity improve the prognosis of the disease <sup>12</sup>.

We already changed it in March according to the original comments of the reviewers. We copy the text from the Review:

### *Environmental factors*

Among environmental factors, the most prominent are dietary habits, physical activity, and socio-economic aspects. Increased calories intake, and consumption of high-sugar and high-fat diets increases the risk of developing not only NAFLD but also conditions such as obesity and type 2 diabetes mellitus <sup>[4, 8, 12]</sup>. Hallsworth, K *et al.* was the first to show an association between sedentary behavior and physical activity levels in patients with NAFLD, finding that these patients were on average more sedentary, walked less and spent less time on physical activity <sup>[13]</sup>. Furthermore, it has been demonstrated that lifestyle interventions in diet and physical activity could improve the disease prognosis <sup>[12]</sup>. Finally, regarding socio-economic aspects, the role of educational level and family economic status in development of NAFLD is still under debate <sup>[4]</sup>.

2.4. Intracellular factors section: Very good.

Thank you very much, we are grateful for your comment.

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