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Previous Member of the "World J. of Biological Chemistry" editorial board, **ID (02810791)**

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To Prof. Timothy R. Kock, Editor-in-Chief of "World J. of Diabetes"
To Prof. Lian-Sheng Ma, Chief Executive Officer

Dear Prof. Koch, Prof. Ma
We would like to thank you for your invitation to submit a revised version of our manuscript (Manuscript NO: 53265) to the World Journal of Diabetes.
Please find in attachment our re-submission which is revised to take into account all the reviewer's criticisms and comments.
Below, you will find our rebuttals to the three independent reviewers.

Conflict of interest statement

All authors have approved this manuscript version and neither of the authors have conflicts of interest related to the submitted manuscript.

We hope you and the reviewers will consider the revised manuscript as acceptable for publication on the World Journal of Diabetes, and we are looking forward to hearing from you,

Sincerely yours, L. Pirola

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Reviewer #1:

Article: The expression of glucose/fatty acid metabolism-related genes in the liver and adipose tissue is altered in male offspring submitted to maternal low-protein diet. My specific queries and comments are below:

We would like to thank the reviewer for her/his insightful comments. Below is a point-by-point response to all the reviewer comments/suggestions, that we believe helped us to substantially improve our manuscript.

Title: • The title is long; Could the authors change the title as few words as possible?

We propose as new title: "Maternal low-protein diet induces persistent expression changes in metabolic genes in male rats". The title counts 13 words as compared to 25 words of the title in the first submission.

Abstract: • The authors should extend the abbreviations, particularly if it is the first time that the term is mentioned.

As requested by the reviewer, all gene names and other acronyms are now also provided in full. Gene names occurring in the abstract, are given in full within the abstract.

• The abstract is under-elaborated and the objectives should be clarified.

Besides adding the gene names, we now open the abstract with the clarifying statement: "Perinatal exposure to a poor nutritional environment predisposes the progeny to the development of metabolic disease at the adult age, both in experimental models and humans." This opening statement puts our work (on experimental rats) into the context of the predisposition by perinatal malnutrition to develop metabolic disease in humans. This important point relative to the relevance to human health, as also requested by reviewer #2, will be further developed in the discussion section.

The abstract's closing statement has also been clarified as follows (new added part in red): "These gene alterations, together with previously described changes in gene expression in skeletal muscle, may account for the metabolic adaptations in response to maternal low protein diet and highlight the occurrence of persistent transcriptional defects in key metabolic genes that may contribute to the development of metabolic alterations during the adult life as a consequence of perinatal malnutrition."

• The abstract should be organized in Background and aims; Material and methods; Results and Conclusion. These sections are conflated.

As suggested by the reviewer, the abstract is now organized with the three sub-sections.

Introduction: • This section would have been strengthened by a thorough review of the literature. There is insufficient information in the Introduction to make a case for why the effects of perinatal low protein diet on the expression of key genes involved in the metabolism of glucose and fatty acid in the liver and adipose tissue of males rats offspring.

We thank you the reviewer for this criticism. We now provide more details to previous literature, citing the epigenetic alterations, as well as the signaling and metabolic changes that are induced by prenatal nutritional restriction. Also to address the point of reviewer #2, we now cite studies in humans.

A new paragraph added in the "Introduction" section links studies in humans and animal models:

"The occurrence of persistent epigenetic alterations has been proposed as one of the mechanisms linking in-utero nutritional deprivation to increased risk of disease in adulthood. Individuals who were prenatally exposed to the Dutch famine during the 1944-45 were shown, 6 decades later, to have lower DNA methylation of the imprinted IGF2 gene in comparison to their siblings not exposed to the famine period (7). Observational studies have suggested a link between poor fetal growth and the development of impaired glucose tolerance at the adult age in both sexes (8), and final evidence that maternal nutrition during gestation affects glucose metabolism in adult life was provided by the observation that an oral glucose load in adults exposed prenatally to the to the Dutch famine lead to higher glycemic concentrations as compared to individuals being born around the same years but not exposed in utero to the 1944-45 famine (9). »

A second paragraph added in the introduction makes the connection between previous studies, showing epigenetic and signaling defects, to our studies addressing low-protein diet induced changes in genes related to the control of metabolism as follows:

"The occurrence of alterations of glycemic control in humans, associated to the observation that perinatal malnutrition induces defects in the insulin signaling pathways in rodent models prompted us to evaluate whether the main metabolic pathways are affected by a low-protein diet administered to dams during pregnancy and lactation."

• The results should be better described. The configurations of the figures should be improved.

We have now revised the results section to give a more streamlined presentation of the results (and overall a revision of the manuscript for improved usage of English language and specified in the next point). Relatively to the presentation of the figures, while in the first submission they were embedded in the manuscript text, they have now been moved to the end of the manuscript, with each figure filling one page.

• The English should be revised.

The manuscript has been thoroughly reviewed by a proficient English speaker (Ms. C. Pirola). A proficiency certificate in English is attached in the submission.

• Reviewer conclusion: Accept but needs minor and major revision.

We appreciate the reviewer's conclusive remarks and we hope that our revision will have improved the manuscript in the reviewer's opinion.

Reviewer #2:

If possible, underline the metabolic impact in humans.

We thank the reviewer for this important and insightful point. To move towards a more inclusive presentation of our data towards the impact of our research (performed in a rat model) to the impact in humans, we have added the following sections in various parts of the manuscript.

Abstract: "Background and aims: Perinatal exposure to a poor nutritional environment predisposes the progeny to the development of metabolic disease at the adult age, both in experimental models and humans."

Introduction: "The occurrence of persistent epigenetic alterations has been proposed as one of the mechanisms linking in-utero nutritional deprivation to increased risk of disease in adulthood. Individuals who were prenatally exposed to the Dutch famine during the 1944-45 were shown, 6 decades later, to have lower DNA methylation of the imprinted IGF2 gene in comparison to their siblings not exposed to the famine period (7). Observational studies have suggested a link between poor fetal growth and the development of impaired glucose tolerance at the adult age in both sexes (8), and final evidence that maternal nutrition during gestation affects glucose metabolism in adult life was provided by the observation that an oral glucose load in adults exposed prenatally to the to the Dutch famine lead to higher glycemic concentrations as compared to individuals being born around the same years but not exposed in utero to the 1944-45 famine (9)."

And:

"The occurrence of alterations of glycemic control in humans, associated to the observation that perinatal malnutrition induces defects in the insulin signaling pathways in rodent models prompted us to evaluate whether the main metabolic pathways are affected by a low-protein diet administered to dams during pregnancy and lactation."

Reviewer #3: No further comments.

We thank the reviewer for having assessed our manuscript without noticing major flaws.