Response to Reviewer 3:

First, we thank to editors and all the reviewers for their effort and the time spent to improve our work and for the insightful comments on our article. Below, we respond to each comment in an itemized fashion, hoping that the related changes to the manuscript are satisfactory, so that the paper can be accepted in its currently revised form.

The modifications made to the manuscript have been marked in the manuscript.

Here is a point-by-point response to the reviewers' comments and concerns.

Comment 1: *The authors should consider adding more information about metformin transporters and receptors in the nervous system.*

Thank you for suggestion. We added the following paragraph in the introduction. Additionally, the references were updated.

Although the precise mechanisms by which metformin is transported in the central nervous system are still unclear, recent in vivo and in vitro studies showed that it can pass the blood-brain barrier and can work by activating certain neurons and neuroglia to pro-vide its desired effects [53,54]. Moreover, metformin transporting has been linked to the equilibrative nucleoside transporter (ENT) family, of which PMAT is a member, and which is present in many bodily tissues, including the brain and central nervous system. [44]. Blood-brain barrier and brain express OCT3, as well [55]. Research on the metformin transporter in the neurological system is currently ongoing, and further investigation is required to clarify the precise processes at play. Nevertheless, the fact that metformin may penetrate the blood-brain barrier and operate neurologically points to possible therapeutic benefits for the central nervous system [53,54].

Comment 2: The authors should consider presenting the discussed item showing the in vivo and in vitro literature results in separate items.

Thank you for the observation and suggestion. Generally, we have structured each of the sections on Alzheimer's' disease, Parkinson's, Huntington's, Epilepsy, and Fragile X syndrome presenting data derived from in vitro studies, animal research, and any type of existing clinical studies (in consecutive, different paragraphs). However, as in some cases the same idea/concept might have been supported by data derived from both in-vivo/in-vitro studies, we did not completely separate those. Following the main focus of our article and the reviewer observation, we considered that changing the title of the manuscript into "Metformin: The winding path from understanding its molecular mechanisms to proving therapeutic benefits in neurodegenerative disorders" would better reflect its content.

Comment 3: - *The authors could create a schematic figure summarizes the main metformin mechanism in T2D and metabolic conditions, showing receptors, transporters, and pathways, and a figure showing the neuroprotective pathways and proprieties in the nervous system, especially in the brain related to memory and cognitive impairments.*

Thank you for your suggestions. We agree on the need of clarifying the concepts and we have added four separate figures regarding metformin's pharmacokinetics, mechanisms involved in glucoregulation, AMPK signaling pathways and signaling pathways involved in neuroprotection:

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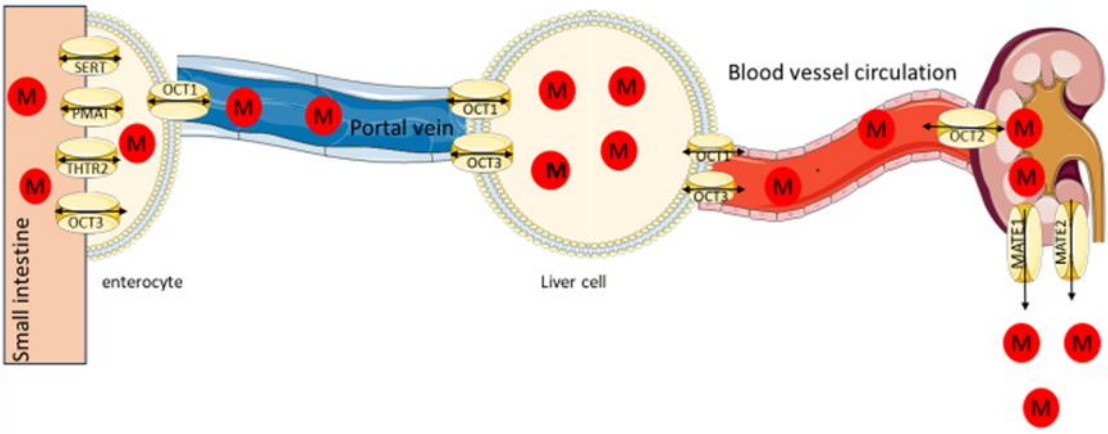


Figure 1. Overview of transporters relevant to metformin pharmacokinetics. MATE multidrug and toxin extrusion transporter, OCT organic cation transporter, PMAT plasma membrane monoamine transporter, SERT serotonin transporter, THTR2 thiamine transporter.

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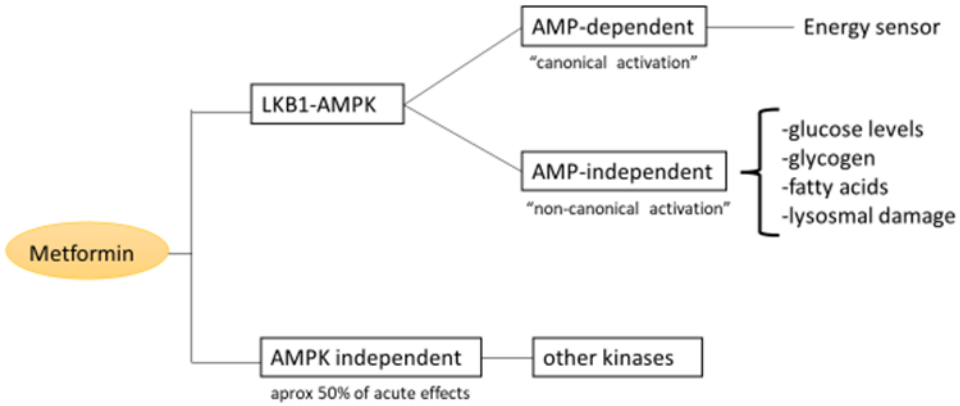


Figure 2. AMPK signaling pathway. AMPK AMP-activated protein kinase, LKB1 Liver kinase B1.

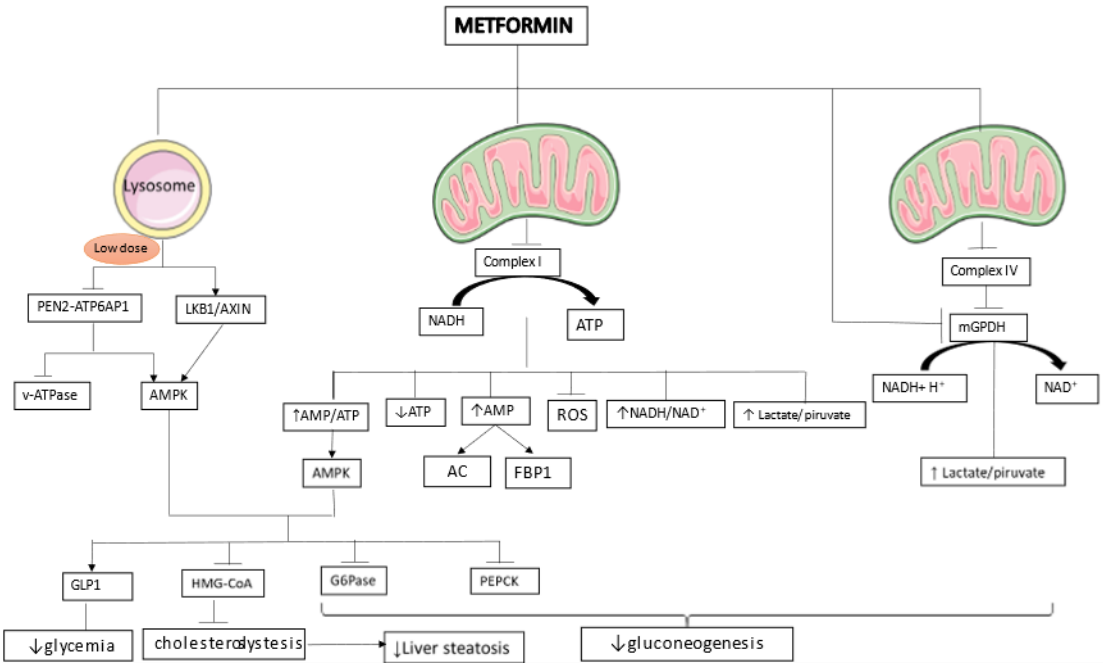


Figure 3. Proposed mechanism of action for metformin Left: Low dose of metformin interferes with the vacuolar H+-ATPase in the lysosomal proton pump (v-ATPase). The interaction between metformin and PEN2 (presenilin enhancer protein 2) forms a complex involving ATP6AP1. The AMPK-dependent model of metformin-induced inhibition of gluconeogenesis is based on the activation of the LKB1–AMPK signalling pathway. Middle: Metformin causes a slight inhibition of mitochondrial respiratory chain complex I in the liver, which results in a moderate reduction in ATP production and an increase in AMP (inhibiting Fruc-tose-1,6-Bisphosphatase Deficiency FBP1 and adenylate cyclase AC) levels in the cells. AMP-activated protein kinase (AMPK) is activated by the metformin-induced rise in the AMP to ATP ratio and increases GLP1 and inactivates/inhibits G6Pase(glucose-6-phosphatase), PEPCK (phosphoenolpyruvate carbox-ykinase) and HMG-CoA (3-hydroxy-3-methylglutaryl coenzyme A). The inhibition of complex I by met-formin is also accompanied by an increased ratio of lactate/pyruvate, and reduced glucose synthesis. Right: Mitochondrial glycerol-3-phosphate dehydrogenase (mGPDH)-dependent. Because metformin directly in-hibits mGPDH, there is a decrease in lactate gluconeogenesis, a reduction in the activity of the glycerol–phosphate shuttle (which moves NADH from the cytosol to the mitochondria), and a rise in the cytosolic redox state (NADH:NAD+).

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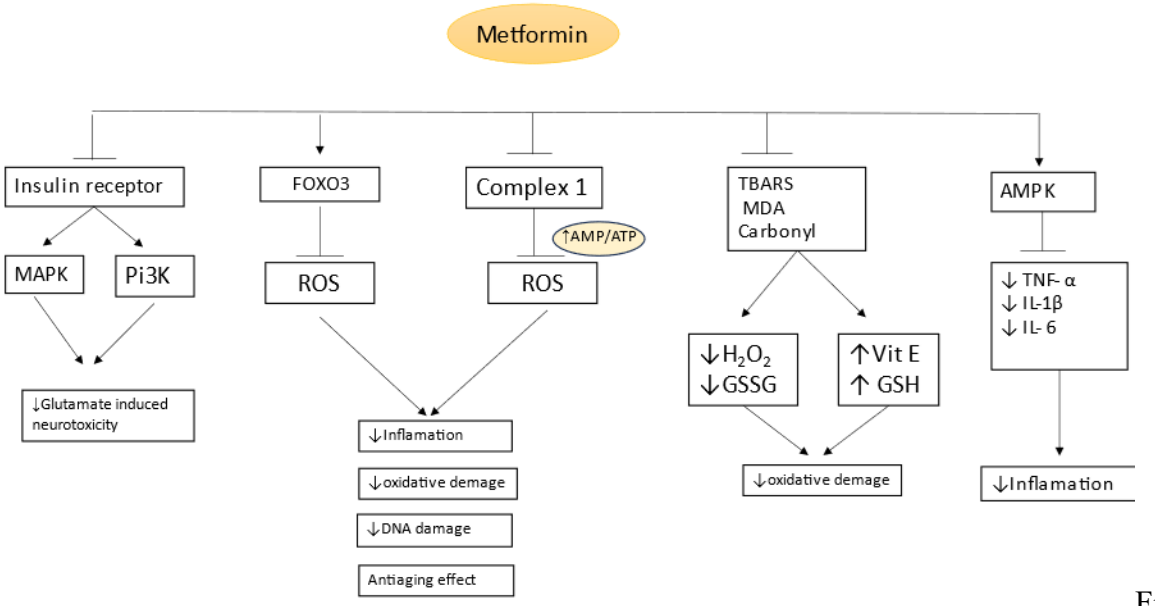


Figure 4. Signaling pathways involved in neuroprotective mechanisms. MAPK mitogen-activated protein kinase, Pi3K phosphatidylinositol 3-kinase, FOXO3 forkhead box O 3, ROS reactive oxygen species, TBARS thiobarbituric acid reactive substances, MDA malondialdehyde, GSSG oxidized form of glutathione, GSH reduced form of glutathione, AMPK adenosine monophosphate-activated protein kinase, TNF-α tumor Necrosis Factor-α, IL-1β interleukin 1 beta, IL-6 interleukin 6.

Comment 4: *Minor comments are highlighted in the attached file.*

Thank you for your careful review of the article and for your comment. We reviewed the entire manuscript for typographical errors and made the needed corrections.