Response to Reviewer 1 Comments

Thank you for your review of the manuscript and your comments. I have made corrections to the text. All corrections in the text are highlighted in green.

The manuscript explores the relationship between NAFLD and metabolism based on previous studies, combs its pathogenesis, and believes that lipid metabolism is an important reason for the occurrence of NAFLD. This view is consistent with the current mainstream research. The manuscript is well-organized and easy to understand. Therefore, the work in this manuscript is a valuable contribution to the field. Thus, this manuscript can be accepted with the following minor changes.

Thank you for the opportunity to review papers entitled: Immune and metabolic cross-links in the pathogenesis of comorbid NAFLD. The manuscript explores the relationship between NAFLD and metabolism based on previous studies, combs its pathogenesis, and believes that lipid metabolism is an important reason for the occurrence of NAFLD. This view is consistent with the current mainstream research. The manuscript is well-organized and easy to understand. Therefore, the work in this manuscript is a valuable contribution to the field. Thus, this manuscript can be accepted with the following minor changes.

Point 1: Keywords: It is recommended to add keywords related to fat or lipid metabolism.

Response 1: Thank you for your comment. I corrected the keywords:

NAFLD, metabolism, lipid metabolism; lipid; fat; innate immune system, pathogenesis

Point 2: Introduction: The content of metabolic associated fatty liver disease (MAFLD) in the third paragraph does not fit the core argument of this article. It is recommended to consider whether to delete it.

Response 2: Thank you for your comment. I deleted this section.

Point 3: The Significance of Metabolic Disorders in the Pathogeness of NAFLD: The manuscript puts forward “Disrupted metabolism of fatty acids is one of the central events in the pathogenesis of NAFLD” in conclusion. The first paragraph is unclear about the relationship between NAFLD, obesity, and lipid metabolism disorder.

Response 3: Thank you for your comment. I added the information and corrected the text

Point 4: The Significance of Metabolic Disorders in the Pathogeness of NAFLD: The second and third paragraphs can be combined into one paragraph.

Response 4: Thank you for your comment. I combined these paragraphs

Point 5: Endothelial Cell Involvement in the Immune System in the Liver: The second and third paragraphs can be combined into one paragraph.

Response 5: Thank you for your comment. I combined these paragraphs

I hope that the corrections I have made have improved the quality of the article.

Response to Reviewer 2 Comments

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Thank you for your review of the manuscript and your comments. I have made corrections to the text. All corrections in the text are highlighted in green.

Point 1: My detailed comments are as follows: 1.In "THE IMPORTANCE OF INNATE IMMUNITY IN THE PATHOGENESIS OF NAFLD" part, the role of Kupffer cells is mainly introduced, while the role of neutrophils, dendritic cells, and NK cells is not mentioned.

Response 1: Thank you for your comment. I have added new information.

Neutrophils, other important participants in the innate immune system, are also involved in the pathogenesis of NAFLD [143]. Given that inflammation is a key event that contributes to the progression of fatty liver dystrophy to NAFLD, these patients show significant neutrophil infiltration into the liver, often accompanied by increased expression of chemokines that promote neutrophil chemotaxis [144].

Neutrophils exhibit cross-links with HSCs. On the one hand, neutrophils activate HSCs through the production of ROS [145-147]. On the other hand, activated HSCs have been shown to support neutrophil survival by producing granulocyte-macrophage colony- stimulating factor (GM-CSF) and IL-15. This may serve as a positive direct loop contributor to liver damage and fibrosis in a high-fat diet [147].

Interestingly, it has been shown that neutrophils in blood patients with NASH had increased expression of receptors reflecting the preparation of neutrophils to migrate into tissue. In addition to preparation for migration, blood neutrophils in NASH were also functionally activated [148]. They were characterized by increased IL-8 production and had more than double the spontaneous oxidative burst. In analyzing these data, it was noted that neutrophils can not only move

from the vascular lumen into extravascular tissues but can also move back into the bloodstream, through a process known as reverse transendothelial migration (rTEM). Reverse transendothelial migration is of interest because of its possible interaction with the immune system [149]. However, its possible role in NAFLD has yet to be studied.

Thus, neutrophils play an important role in the development of inflammation and liver fibrosis [150]. On the other hand, neutrophils contribute to the spontaneous resolution of inflammation and liver fibrosis. Acting via miR-223, neutrophils act as resolving effector cells that induce the transition of proinflammatory macrophages to a restorative phenotype by suppressing NLRP3 inflammasome expression [151]. Another study in a diet-induced NASH mouse model also showed a phase-dependent contrasting role of neutrophils as triggers and pro-resolutive mediators of liver damage and fibrosis [150]. In addition to these findings, miR-223 was shown to be elevated in hepatocytes from HFD-treated mice and patients with NASH, which may be due to the fact that miR-223 can be transferred from neutrophils via the exosome. Moreover, miR-223 in hepatocytes acts as an anti-inflammatory molecule, directly affecting several inflammatory genes [152].

Thus, neutrophils play a complex multifaceted role in the pathogenesis of NAFLD, which is a promising topic for further research.

Liver DCs are a heterogeneous population of hepatic sinusoidal antigen-presenting cells [153, 154]. DCs exist in mature or immature states and undergo maturation when exposed to immune or inflammatory signals such as microbial products and proinflammatory cytokines. DCs are involved in maintaining immune homeostasis and liver tolerance by promoting CD8+ T-cell elimination, as well as secreting anti-inflammatory cytokines that maintain the quiescent HSC state and promote TLR4 refractoriness to LPS. In addition, DCs regulate the number and activity of cells involved in the development of fibrosis and may play a role in the regression of liver fibrosis [155]. Dendritic cells can contribute to liver fibrosis regression by activating metalloproteinases and also contribute to the homeostasis of NK cells, which are mainly antifibrogenic [154].

Natural killer cells are a heterogeneous multifunctional population of lymphoid cells located inside the sinusoidal space, where they can attach to endothelium and Kupffer cells [156]. A key factor determining the activity of these cells in NASH is their metabolic reprogramming.

Liver NK cells are part of the innate immune system and may play an important role in NAFLD. However, the regulation and function of NK cells in NAFLD remains controversial because of their different involvement at different stages of the disease. On the one hand, NK cells are active and may be useful in the early stages of fibrosis, when they contribute to TRAIL-mediated hepatic stellate cell death. On the other hand, NK cell involvement becomes detrimental

when they lose their antitumor capacity, which may contribute to disease progression in later stages [156]. Indeed, metabolic reprogramming of natural killer cells in obesity limits the antitumor response, which is known as "metabolic paralysis"[157]. Overload of NK cells with lipids absorbed from the environment in obesity leads to metabolic defects that cause inhibition of the cytotoxic mechanism, resulting in loss of antitumor functions [157]. Overall, the available data suggest a possible therapeutic potential for the regulation of NK cell function, which is a promising topic for further research.

Point 2: Large text description is not conducive for readers to understand the content of the article. Please draw a schematic diagram according to the content of each part.

Response 2: Thank you for your comment. I have added new 3 figures.

Point 3: Could you add some prospects for NAFLD research?

Response 3: Thank you for your comment. I have added new information.

1.Another immunometabolic link between the gut microbiota and NAFLD, related to short-chain fatty acids (SCFAs), should also be noted [104, 105]. SCFAs are formed by the gut microbiota during the fermentation of non-digestible fibers such as resistant starch, cellulose, and pectin [106]. SCFAs are used by colonic mucosal epithelial cells as an energy substrate, are involved in the regulation of a number of processes in the intestinal wall or enter the portal bloodstream, and may be involved in the formation of immunometabolic connections with other organs [107].

A growing body of evidence strengthens the understanding of the importance of SCFAs in inflammation. SCFAs act via receptors associated with the G-protein GPR43 and GPR41, also known as free fatty acid receptor (FFA)2 and FFA3, respectively [108-111]. In addition, SCFAs realize their action through inhibition of histone deacetylase (HDAC) [112, 113].

Butyrate is well known for its anti-inflammatory properties and is of great clinical interest [107, 114, 115]. Through HDAC3 inhibition, butyrate can induce a metabolic switch of macrophages toward an anti-inflammatory M2 phenotype [112, 113].

SCFAs are also known to affect the differentiation, recruitment and activation of neutrophils, dendritic cells, macrophages and monocytes as well as T cells [116, 117]. Butyrate is involved in the regulation of dendritic cell differentiation derived from human monocytes, keeping dendritic cells in the immature stage [118].

In addition to their involvement in inflammation, SCFAs regulate lipid metabolism in the liver. Butyrate levels have been shown to decrease in NAFLD patients and mice with decreased estrogen levels, with butyrate administration attenuating liver steatosis [119]. Studies in rats fed HFD have shown that butyrate increases β-oxidation of fatty acids, inhibits lipid synthesis and suppresses nuclear factor-kappa B and inflammation [120, 121]. The addition of sodium butyrate protects mice from developing NASH. It is important to note

that the metabolic role of SCFAs in liver function is rather complex [122]. In addition to attenuating hepatic steatosis, acetate, another SCFAs derived from the microbiota, may conversely promote hepatic lipogenesis after excessive fructose intake [123, 124].

2. The pathogenesis of NAFLD is an important target for further research, among which immunometabolic cross-linkages can be considered as one of the promising directions. Immunometabolic regulation of cells and intercellular connections at different stages of liver disease development can be a significant target for therapeutic intervention. In addition, the immune and metabolic axes that link the liver to other organs are also of research and clinical interest. There is a growing understanding that the gut microbiota is an important participant in immune and metabolic processes not only in the gut, but also in other organs. There is also interest in information about the cross-linkages of lipid-transport function and innate immunity, which have evolutionarily conservative roots and link a number of diseases that mutually influence their natural history.
In summary, NAFLD is a complex multifaceted problem whose keys are still unknown to clinicians and researchers, but a better understanding of metabolic and immune cross-linkages will improve patient diagnosis and treatment approaches.

I hope that the corrections I have made have improved the quality of the article.