

Cholecystectomy inhibits fasting hepatic fatty acid oxidation in mice

Corresponding Author: Dr Yongsheng Chen

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

This paper explores the mechanism of cholecystectomy (XGB)- induced changes of some parameters of lipid and glucose metabolism in C57BL/6 mice. Studies were performed using metabolomics and quantitative RT-PCR gene expression techniques in serum, liver and intestinal specimens. The main results showed that XGB by increasing fasting hepatic bile acid levels, suppressed genes involved in fatty acid oxidation (FAO) through inhibition of PPAR α . XGB mice also had increased hepatic fat content, with changes in metabolomic profiles. FAO genes were transcriptional targets of the peroxisome proliferator-activated receptor α (PPAR α). Authors also showed that blocking intestinal bile acid reabsorption increased hepatic fatty acid levels and improved metabolic parameters in XGB mice. These findings were consistent with a metabolic role of the gallbladder in coordinating PPAR α -regulated fatty acid metabolism in the liver. The paper is sound, well written, but needs some significant changes for improvement.

Please check.

1. The title should include "...in mice".
2. The terms "in metabolic health" is ambiguous. Please, see line 20 and elsewhere. I suggest "...in some parameters of glucose, lipid and bile acid metabolism".
3. Introduction, line 37. The term "cholecystitis" should be "gallstone disease".
4. I suggest summarizing the results section entitled "Cholecystectomy increased fasting hepatic BA concentration", lines 258 -294. Also, you might move Fig 5 A to Supplements, as it does not add to the clarity of the manuscript.
5. The Discussion needs attention. This section should be fully reworked based on the scheme:
 - a. I suggest starting with a summary of the most significant original results of the study.
 - b. How these novel findings position within current literature
 - c. Strengths and limitations of the study.
 - d. Conclusions.

Reviewer #2

(Remarks to the Author)

Qi et al present a manuscript that evaluated the effects of cholecystectomy on liver and bile acid metabolism. The authors present a thorough examination with a large amount of data, including the finding that steatosis after cholecystectomy may disrupt fasting-induced fatty acid oxidation and fat use in the liver. The authors can improve the manuscript with some additional data and discussion of the results.

Major Comments

1) The authors conclude that altered enterohepatic circulation is responsible for the increased hepatic bile acids, rather than upregulation of synthesis via CYP7A1 or other BA enzymes. However, Supplemental Fig 1C shows that the top upregulated pathway identified by KEGG is bile acid synthesis. The authors may consider adding a sentence to interpret these results -

this pathway was identified due to the presence of excess bile acids, but may not be accurate in estimating bile acid synthesis itself. Similarly, cholesterol metabolism

2) The results state that GSK treatment "markedly reduced BA levels" but this data was not shown. The authors should provide confirmatory data showing how distribution of the BA pool changes with GSK in XGB mice.

3) The methods state that food intake was recorded but this data was not shown. Food intake graphs should be shown for all separate mouse experiments.

Minor Comments

1) The authors refer to chronobiology throughout the manuscript, though this is typically reserved for the specific study of circadian rhythms (clock genes, clock-controlled genes, and transcription factors) and how they produce 24-hr rhythms. BA cycling occurs multiple times per day in sync with feeding-fasting and thus, is not a true circadian output. The authors can consider replacing some of these instances with "diurnal regulation", "feeding-fasting cycles", or other more accurate terms.

Reviewer #3

(Remarks to the Author)

Title: Cholecystectomy inhibits fasting hepatic fatty acid oxidation

The current manuscript investigates the metabolic consequences of cholecystectomy in C57BL/6 mice, with a particular focus on the suppression of hepatic fatty acid oxidation (FAO) due to disrupted bile acid (BA) dynamics. The authors attribute this metabolic shift to bile acid-mediated antagonism of PPAR α signaling and propose that pharmacologic inhibition of bile acid reuptake may offer therapeutic benefit. While the experimental approach is methodologically sound and employs a broad array of tools including metabolomics, transcriptomics, and pharmacologic intervention, the overall findings are incremental and largely reaffirm previously reported phenomena. Several points merit further attention:

- Novelty and Scope:

The central concept that cholecystectomy alters enterohepatic BA circulation with downstream metabolic consequences is not novel. Several studies (e.g., Huh et al., 2023; Luo et al., 2023) have already established links between gallbladder removal and increased risk of metabolic syndrome or NAFLD. Moreover, the regulatory roles of BA signaling on hepatic metabolism through nuclear receptors such as FXR and PPAR α are well-documented (e.g., Mohanty et al., 2024; Preidis et al., 2017). The manuscript provides a mechanistic confirmation of these established pathways in a cholecystectomy mouse model but lacks the conceptual novelty or broad biological insight required for publication in a journal of the scope of Communications Biology.

- Mechanistic Claims Require Caution:

While the repression of PPAR α targets by BA accumulation is convincingly demonstrated, the authors' emphasis on circadian disruption lacks supporting data. No time-course analyses or clock gene expression data are provided to substantiate the chronobiology claims. This weakens one of the core interpretative frameworks of the study.

- Translational Relevance is Limited:

The findings are primarily restricted to a murine model and are not supported by any human data. Given interspecies differences in BA composition, metabolism, and regulatory networks, extrapolating these results to human physiology should be done with caution. This limits the translational impact of the work.

- Pharmacologic Intervention:

The use of GSK2330672 (ASBT inhibitor) adds value by pointing toward a potential therapeutic strategy; however, the translational implications remain speculative in the absence of long-term studies or validation in human-relevant models.

- Statistical and Experimental Power:

Several experiments are conducted with small sample sizes ($n=3-6$), reducing confidence in the reproducibility and robustness of the conclusions. This is particularly concerning given the inherent variability in metabolic studies.

- Presentation and Redundancy:

The manuscript would benefit from more concise and focused writing. Several sections reiterate the same findings, and the flow between results and interpretation is occasionally disrupted by overextended discussion points.

Summary and Recommendation:

In summary, this study is methodologically rigorous and presents a well-executed exploration of how cholecystectomy impacts hepatic fatty acid oxidation through bile acid-mediated repression of PPAR α signaling. However, the findings, while supportive of existing models, represent an incremental contribution to the field. The mechanistic link between bile acid accumulation and PPAR α inhibition has been previously described in both experimental and clinical contexts.

To enhance the impact and suitability of the manuscript for a broad-interest journal such as Communications Biology, the authors are encouraged to:

1. Integrate human data—such as clinical metabolic profiles or liver biopsies from cholecystectomized patients—to strengthen translational relevance.
2. Incorporate circadian analysis—including time-course studies of gene expression or BA profiles—to substantiate claims

regarding chronobiological disruption and circadian disturbances.

3. Clarify novelty by distinguishing how their findings significantly advance beyond prior work (e.g., Sinal et al., 2001; Mohanty et al., 2024; Arab et al., 2017).

4. Explore systemic metabolic effects, such as cross-talk between liver, gut, and adipose tissue, to broaden the scope and appeal to a wider biological audience.

Without these enhancements, the manuscript may be more appropriately positioned in a specialized journal focused on hepatobiliary physiology, metabolic disease, or gastrointestinal biology.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

The revised version of the paper has substantially improved its presentation; however, there are some aspects that require attention in the discussion, which should focus on the study's results while avoiding excessive speculation. I suggest the following:

1. Remove the paragraphs in the Conclusion. They are redundant and overly speculative.

2. Avoid addressing the role of cholecystectomy in the management of asymptomatic and symptomatic cholelithiasis, a highly controversial topic in clinical medicine that deviates from the central theme of this basic science work.

3. Change the first paragraph of the discussion: the term "greater digestive power" is confusing.

Reviewer #3

(Remarks to the Author)

I have carefully reviewed the revised version of the manuscript entitled "Cholecystectomy inhibits fasting hepatic fatty acid oxidation in mice" by Dr. Chen and colleagues. The authors have addressed all the points raised in the previous round of review in a thorough and satisfactory manner. The responses to the reviewers' comments are appropriate, and the additional data and clarifications provided have strengthened the manuscript substantially. The discussion has been improved and now provides a balanced interpretation of the findings within the context of existing literature.

I did not identify any remaining major issues that need to be addressed before publication. Minor editorial polishing may be handled during the journal's production

made.

In cases where reviewers are anonymous, credit should be given to 'Anonymous Referee' and the source.

The images or other third party material in this Peer Review File are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder.

To view a copy of this license, visit <https://creativecommons.org/licenses/by/4.0/>

Reviewers' comments:

Reviewer #1 (Remarks to the Author):

This paper explores the mechanism of cholecystectomy (XGB)- induced changes of some parameters of lipid and glucose metabolism in C57BL/6 mice. Studies were performed using metabolomics and quantitative RT-PCR gene expression techniques in serum, liver and intestinal specimens. The main results showed that XGB by increasing fasting hepatic bile acid levels, suppressed genes involved in fatty acid oxidation (FAO) through inhibition of PPAR α . XGB mice also had increased hepatic fat content, with changes in metabolomic profiles. FAO genes were transcriptional targets of the peroxisome proliferator-activated receptor α (PPAR α). Authors also showed that blocking intestinal bile acid reabsorption increased hepatic fatty acid levels and improved metabolic parameters in XGB mice. These findings were consistent with a metabolic role of the gallbladder in coordinating PPAR α -regulated fatty acid metabolism in the liver. The paper is sound, well written, but needs some significant changes for improvement.

Please check.

1. The title should include "...in mice".
2. The terms "in metabolic health" is ambiguous. Please, see line 20 and elsewhere. I suggest "...in some parameters of glucose, lipid and bile acid metabolism".
3. Introduction, line 37. The term "cholecystitis" should be "gallstone disease".

Response: Thanks a lot for these constructive comments. We have carefully revised the manuscript to address all points raised, including the title, terminology, and specific wording as suggested. The changes have been implemented throughout the text.

4. I suggest summarizing the results section entitled "Cholecystectomy increased fasting hepatic BA concentration", lines 258 -294. Also, you might move Fig 5 A to Supplements, as it does not add to the clarity of the manuscript.

Response: We thank the reviewer for these helpful suggestions. Regarding the summary of the results section "Cholecystectomy increased fasting hepatic BA concentration", we have now condensed this paragraph to improve its conciseness and flow, as recommended. Concerning Fig 5A, we agree that it is not essential for the main narrative. We have therefore moved it to the Supplementary Information (now Supplementary Fig.2) to streamline the main figures while retaining the data for interested readers.

5. The Discussion needs attention. This section should be fully reworked based on the scheme:
 - a. I suggest starting with a summary of the most significant original results of the study.
 - b. How these novel findings position within current literature
 - c. Strengths and limitations of the study.
 - d. Conclusions.

Response: We sincerely thank the reviewer for the constructive suggestion to restructure the Discussion. Following the recommended framework, we have thoroughly reorganized this section to begin with a concise summary of our key findings linking the clinical paradox to our elucidated mechanism, explicitly discuss the study's strengths and its limitation regarding the murine model with an interpretation of our human metabolomics data, and conclude with clear take-home messages. We believe these revisions have significantly improved the logical flow and scholarly impact of the Discussion.

Reviewer #2 (Remarks to the Author):

Qi et al present a manuscript that evaluated the effects of cholecystectomy on liver and bile acid metabolism. The authors present a thorough examination with a large amount of data, including the finding that steatosis after cholecystectomy may disrupt fasting-induced fatty acid oxidation and fat use in the liver. The authors can improve the manuscript with some additional data and discussion of the results.

Major Comments

1) The authors conclude that altered enterohepatic circulation is responsible for the increased hepatic bile acids, rather than upregulation of synthesis via CYP7A1 or other BA enzymes. However, Supplemental Fig 1C shows that the top upregulated pathway identified by KEGG is bile acid synthesis. The authors may consider adding a sentence to interpret these results - this pathway was identified due to the presence of excess bile acids, but may not be accurate in estimating bile acid synthesis itself. Similarly, cholesterol metabolism

Response: In Supplemental Fig 1C, KEGG pathway analysis identified "bile acid synthesis" as an upregulated pathway. It is important to note that pathway analysis is based on gene expression changes and may reflect the overall altered bile acid metabolic state, including feedback responses to the elevated hepatic bile acid pool, rather than directly demonstrating an increase in de novo synthesis. Our conclusion that altered enterohepatic circulation is the primary driver is supported by the direct measurements of hepatic bile acid levels and the fact that the key synthetic enzyme CYP7A1 did not show a corresponding increase at the protein or functional activity level. Therefore, we interpret the KEGG result as a secondary consequence of bile acid accumulation rather than evidence of upregulated synthesis as the initiating cause.

Additionally, we note that the comment on cholesterol metabolism appears to be an incomplete sentence. Could the reviewer please elaborate on this point so that we may address it fully?

2) The results state that GSK treatment "markedly reduced BA levels" but this data was not shown. The authors should provide confirmatory data showing how distribution of the

BA pool changes with GSK in XGB mice.

Response: Thank you for your valuable comments and suggestions. We apologize for any confusion regarding the data on bile acid levels and the efficacy of GSK treatment. Upon reevaluation, we realize that our initial presentation may have given the impression that these findings were part of our experimental results, whereas they were actually derived from the literature. To clarify, the statement regarding the marked reduction of bile acid levels by GSK treatment is based on the findings reported in the literature. Specifically, we refer to the studies (PMID 33965587 and 36737039) in the text, which have demonstrated the significant impact of GSK on bile acid levels. We have revised the relevant section of our manuscript to more accurately reflect this by explicitly citing these studies and stating that the data on bile acid levels is from the literature.

3) The methods state that food intake was recorded but this data was not shown. Food intake graphs should be shown for all separate mouse experiments.

Response: We thank the reviewer for raising this important point. We sincerely apologize for the oversight in our methods description. Food intake was not systematically quantified for the experiments presented, and we will correct this inaccuracy in the revised methods section.

Minor Comments

1) The authors refer to chronobiology throughout the manuscript, though this is typically reserved for the specific study of circadian rhythms (clock genes, clock-controlled genes, and transcription factors) and how they produce 24-hr rhythms. BA cycling occurs multiple times per day in sync with feeding-fasting and thus, is not a true circadian output. The authors can consider replacing some of these instances with "diurnal regulation", "feeding-fasting cycles", or other more accurate terms.

Response: We thank the reviewer for this insightful comment and for highlighting the important distinction. We agree that the term "chronobiology" is more precisely applied to rhythms driven by the core circadian clock. As the reviewer correctly points out, the cycling of bile acids in our study is more directly aligned with feeding-fasting cycles. We have therefore revised the manuscript accordingly and replaced "chronobiology" with more accurate terms "feeding-fasting cycles" throughout the text. This change has improved the precision of our scientific language.

Reviewer #3 (Remarks to the Author):

Title: Cholecystectomy inhibits fasting hepatic fatty acid oxidation

The current manuscript investigates the metabolic consequences of cholecystectomy in C57BL/6 mice, with a particular focus on the suppression of hepatic fatty acid oxidation (FAO) due to disrupted bile acid (BA) dynamics. The authors attribute this metabolic shift

to bile acid-mediated antagonism of PPAR α signaling and propose that pharmacologic inhibition of bile acid reuptake may offer therapeutic benefit. While the experimental approach is methodologically sound and employs a broad array of tools including metabolomics, transcriptomics, and pharmacologic intervention, the overall findings are incremental and largely reaffirm previously reported phenomena. Several points merit further attention:

- Novelty and Scope:

The central concept that cholecystectomy alters enterohepatic BA circulation with downstream metabolic consequences is not novel. Several studies (e.g., Huh et al., 2023; Luo et al., 2023) have already established links between gallbladder removal and increased risk of metabolic syndrome or NAFLD. Moreover, the regulatory roles of BA signaling on hepatic metabolism through nuclear receptors such as FXR and PPAR α are well-documented (e.g., Mohanty et al., 2024; Preidis et al., 2017). The manuscript provides a mechanistic confirmation of these established pathways in a cholecystectomy mouse model but lacks the conceptual novelty or broad biological insight required for publication in a journal of the scope of Communications Biology.

- Mechanistic Claims Require Caution:

While the repression of PPAR α targets by BA accumulation is convincingly demonstrated, the authors' emphasis on circadian disruption lacks supporting data. No time-course analyses or clock gene expression data are provided to substantiate the chronobiology claims. This weakens one of the core interpretative frameworks of the study.

Response: We thank the reviewer for this insightful comment, which allows us to clarify a key aspect of our study. We agree that our manuscript does not provide data demonstrating a disruption of the core circadian clock. We apologize for any lack of clarity in our original phrasing. The central point we intended to make is that cholecystectomy disrupts the physiological, feeding-rhythm-driven dynamics of bile acid (BA) enterohepatic circulation, which operates within the framework of the light-dark cycle but is distinct from the core transcriptional clock.

The experimental design specifically Targets Feeding-Fasting Rhythms. As described in the manuscript, our experiments were conducted under a 12-h light/12-h dark cycle with feeding restricted to the dark (active) phase and fasting enforced in the light (resting) phase. This standard protocol aligns the metabolic challenge of nutrient processing with the animal's intrinsic active period. The design specifically investigates how the timing of food intake (the feeding-fasting rhythm) governs BA flux, a rhythm that is directly abolished by the removal of the gallbladder.

The gallbladder coordinates feeding rhythms, not the core clock: The primary function of the gallbladder is to store and concentrate bile during the fasting state and to eject it upon feeding. It thus acts as a "gatekeeper" that synchronizes BA delivery with the anticipatory rhythm of food intake. Our data show that cholecystectomy eliminates this pulsatile release,

leading to a constant trickle of BA into the intestine and the subsequent fasted-state hepatic BA accumulation we observed. This represents a direct disruption of feeding-entrained BA rhythm.

Following the reviewer's valuable suggestion, we have revised the discussion throughout the manuscript to rectify any imprecise terminology. We have replaced references to "circadian disruption" with more accurate terms such as "feeding-fasting rhythm," "diurnal rhythm of bile acid circulation," and "feeding-entrained metabolic rhythm." We have also explicitly stated that the observed effects are a consequence of disrupted BA dynamics linked to the feeding cycle, which is a separate issue from the regulation of the core molecular clock.

We believe these clarifications and textual corrections significantly strengthen the manuscript and accurately frame our central interpretation: that the gallbladder is a critical coordinator of feeding-cycle-driven metabolic rhythms and its removal disrupts hepatic metabolic homeostasis through this mechanism.

Thank you again for prompting us to improve the precision and impact of our work.

- Translational Relevance is Limited:

The findings are primarily restricted to a murine model and are not supported by any human data. Given interspecies differences in BA composition, metabolism, and regulatory networks, extrapolating these results to human physiology should be done with caution. This limits the translational impact of the work.

Response: We sincerely thank the reviewer for this critical comment. We fully agree with the reviewers that extrapolating findings from mouse models to human physiology requires great caution. We have completed metabolomic analysis of peripheral blood samples from a human clinical cohort and have integrated the relevant results and discussions as important supplementary content into the manuscript. Specifically, we found that key bile acids (including Cholic acid, Deoxycholic acid, and their glycine conjugates) displayed a clear upward trend in the cholecystectomy group compared to controls, as visualized in the new bar graphs (Supplementary Fig.5C). Concurrently, we observed downward trends in several fatty acids and acylcarnitines (Supplementary Fig.5D-E). Although these changes did not reach formal statistical significance, likely due to the substantial inter-individual variability inherent in clinical populations and the buffering capacity of systemic circulation, the consistent directional trends across multiple metabolites are noteworthy. These human data offer clinical corroboration for the biological plausibility of the pathway we identified. The revised text now integrates these clinical trends, presenting a more complete perspective that connects our mechanistic insights from the mouse model with observational data from human patients.

- Pharmacologic Intervention:

The use of GSK2330672 (ASBT inhibitor) adds value by pointing toward a potential therapeutic strategy; however, the translational implications remain speculative in the absence of long-term studies or validation in human-relevant models.

Response: We thank the reviewer for raising this important point regarding the translational implications of our ASBT inhibitor data. We agree that the long-term therapeutic efficacy in patients requires further validation. In our study, the primary objective of using GSK2330672 was not to present a fully developed therapy, but to serve as a decisive mechanistic probe. By administering the ASBT inhibitor, we aimed to perform a direct functional test of our central hypothesis: that the elevated enterohepatic bile acid load following cholecystectomy is the key driver of the observed metabolic dysfunction. The robust reversal of hepatic FAO suppression and metabolic disorders upon treatment provides compelling functional evidence that the BA-PPAR α -FAO axis is a central and targetable pathway in this pathophysiology. Furthermore, while we acknowledge that clinical translation is a future goal, these findings move the potential therapeutic strategy from a speculative idea to a mechanism-based proposition. They establish a strong scientific rationale for focusing on ASBT inhibition as a viable approach worthy of further investigation.

In the revised-manuscript, we have toned down the language in the discussion to avoid overstating immediate clinical applicability. We have included in the Discussion a clear statement on the limitation stemming from the lack of long-term studies or validation in human-relevant models. Although our preclinical model and short-term intervention provide crucial proof-of-concept evidence, their therapeutic applicability needs further assessment in long-term clinical settings. These results is a foundational insight that warrants future investigation in long-term studies and more clinically relevant models to fully assess their therapeutic potential. Our work, by establishing a causal mechanism and identifying a druggable target within it, provides a critical first step on the path toward any future translation.

- Statistical and Experimental Power:

Several experiments are conducted with small sample sizes ($n=3-6$), reducing confidence in the reproducibility and robustness of the conclusions. This is particularly concerning given the inherent variability in metabolic studies.

Response: We appreciate the reviewer's attention to the important issue of sample size, which is critical for ensuring the robustness of scientific findings. We acknowledge that only a very small subset of experiments in our study was conducted with $n=3$. The overwhelming majority of the experimental data, which form the foundation of our central conclusions, were performed with robust group sizes of $n=5$ or greater. This includes all key functional and interventional assays. Furthermore, all LC-MS-based metabolomic analyses, which are core to our findings, were conducted with a minimum of $n=6$ biologically independent samples per group, a sample size that is standard and well-powered in the field for such comprehensive profiling. Therefore, the primary mechanistic insights of our manuscript are supported by datasets with adequate replication, and we are confident in their robustness. Moving forward, we are committed to increasing sample sizes

in our future experimental designs to further enhance the reliability and generalizability of our research.

- Presentation and Redundancy:

The manuscript would benefit from more concise and focused writing. Several sections reiterate the same findings, and the flow between results and interpretation is occasionally disrupted by overextended discussion points.

Response: We sincerely thank the reviewer for the helpful suggestion to improve our manuscript's clarity. We have thoroughly revised the text to make it more concise and focused. Specifically, we have removed repeated findings and reorganized the results to present data more logically. Extended discussions have been moved to the discussion section. We believe these changes have significantly improved the flow and readability of our paper.

Summary and Recommendation

In summary, this study is methodologically rigorous and presents a well-executed exploration of how cholecystectomy impacts hepatic fatty acid oxidation through bile acid-mediated repression of PPAR α signaling. However, the findings, while supportive of existing models, represent an incremental contribution to the field. The mechanistic link between bile acid accumulation and PPAR α inhibition has been previously described in both experimental and clinical contexts.

To enhance the impact and suitability of the manuscript for a broad-interest journal such as Communications Biology, the authors are encouraged to:

1. Integrate human data—such as clinical metabolic profiles or liver biopsies from cholecystectomized patients—to strengthen translational relevance.

Response: We have completed metabolomic analysis of peripheral blood samples from a human clinical cohort and have integrated the relevant results and discussions as important supplementary content into the discussion section of the manuscript. Our analysis of the clinical cohort reveals consistent trends that align with the murine model. Key bile acids were elevated, while certain fatty acids and acylcarnitines were lower in cholecystectomy patients. These findings, now included in the revised manuscript, offer clinical support for the biological plausibility of the metabolic pathway we have identified.

2. Incorporate circadian analysis—including time-course studies of gene expression or BA profiles—to substantiate claims regarding chronobiological disruption and circadian disturbances.

Response: Thank you for prompting us to improve the precision and impact of our work. As mentioned before, we have revised the discussion throughout the manuscript to rectify any imprecise terminology. We have replaced references to "circadian disruption" with more accurate terms such as "feeding-fasting rhythm." We have also explicitly stated that the observed effects are a consequence of disrupted BA dynamics linked to the feeding cycle, which is a separate issue from the regulation of the core molecular clock. We believe these clarifications and textual corrections significantly strengthen the manuscript and accurately frame our central interpretation: that the gallbladder is a critical coordinator of

feeding-cycle-driven metabolic rhythms and its removal disrupts hepatic metabolic homeostasis through this mechanism.

3. Clarify novelty by distinguishing how their findings significantly advance beyond prior work (e.g., Sinal et al., 2001; Mohanty et al., 2024; Arab et al., 2017).

Response: We thank the reviewer for the opportunity to clarify the novelty of our work. The important advancement of this study lies in organically integrating and advancing the bile acid-PPAR α antagonism mechanism discovered by Sinal et al., the systemic regulatory paradigm of bile acids proposed by Mohanty et al., and the therapeutic prospects of targeting bile acids reviewed by Arab et al. into a specific disease model. Specifically, Sinal's team revealed the molecular mechanism by which bile acids antagonize PPAR α by impairing coactivator recruitment; Mohanty et al. expanded the macro-level perspective of bile acid diversity and systemic regulatory functions; and Arab et al. reviewed the potential of targeting bile acid pathways for treating metabolic liver diseases. Our study not only validates these prior findings but also, for the first time, connects the complete causal chain of "surgical intervention - altered bile acid kinetics - multi-tissue metabolic dysregulation - sustained PPAR α functional suppression - onset of metabolic syndrome" through a post-cholecystectomy metabolic syndrome model. Thereby, we establish Sinal's molecular mechanism as a core pathophysiological event, ground Mohanty's macro-paradigm in a specific disease context, and provide experimental evidence guided by precise pathological mechanisms for the therapeutic vision proposed by Arab. Furthermore, by systematically analyzing metabolic profiles in multiple tissues, including peripheral blood, intestine, feces, and liver. We constructed a comprehensive map of systemic metabolic network remodeling triggered by cholecystectomy, further enhancing the completeness and persuasiveness of our mechanistic explanation. In summary, the present study elevates the PPAR α antagonism mechanism from a molecular observation to a core pathophysiological driver; builds a bridge between the systemic bile acid regulatory paradigm and a concrete metabolic disease model; and comprehensively reveals the characteristics of post-operative metabolic network remodeling through multi-tissue metabolic analysis.

4. Explore systemic metabolic effects, such as cross-talk between liver, gut, and adipose tissue, to broaden the scope and appeal to a wider biological audience.

Without these enhancements, the manuscript may be more appropriately positioned in a specialized journal focused on hepatobiliary physiology, metabolic disease, or gastrointestinal biology.

Response: We thank the reviewer for this excellent suggestion to explore the systemic metabolic crosstalk, which has significantly broadened the perspective of our manuscript. Following this advice, we have re-analyzed our existing data and extensively revised the discussion to highlight the inter-organ communication between the liver, gut, and adipose tissue. Specifically, we supplemented data about how the accelerated EHC alters the gut microbial community (Supplementary Fig. 4G-H). Besides, we have incorporated the

discussion on how the observed systemic hyperlipidemia (elevated serum TG & VLDL, Fig. 1B, C) and insulin resistance (Fig. 1D-I) likely originate from the convergence of hepatic metabolic disruption (e.g., suppressed FAO, steatosis) and adipose tissue dysfunction, potentially driven by altered BA signaling. BA as Systemic Messengers: The role of bile acids as endocrine molecules mediating this crosstalk is now a central theme in our revised discussion, linking our molecular findings to the broader metabolic phenotype. we acknowledge the limited depth of our investigation into inter-organ crosstalk as a key aspect we address in the discussion. While our study identifies a potential link between altered hepatic function and systemic metabolism, it offers a foundational framework rather than a definitive mapping of the underlying communication pathways. Future research needs to resolve the major unanswered question, specifically the precise hierarchy and dynamics of signals among the liver, gut, and adipose tissue.

REVIEWERS' COMMENTS:

Reviewer #1 (Remarks to the Author):

The revised version of the paper has substantially improved its presentation; however, there are some aspects that require attention in the discussion, which should focus on the study's results while avoiding excessive speculation. I suggest the following:

1. Remove the paragraphs in the Conclusion. They are redundant and overly speculative.

Re: Thanks for your help. The Conclusion paragraph has been removed in this revision.

2. Avoid addressing the role of cholecystectomy in the management of asymptomatic and symptomatic cholelithiasis, a highly controversial topic in clinical medicine that deviates from the central theme of this basic science work.

Re: We thank the reviewer for this valuable comment. We agree that the role of cholecystectomy is a complex clinical issue beyond the scope of our basic research. To ensure the manuscript remains focused on its central theme, we have deleted the corresponding segment from the Discussion.

3. Change the first paragraph of the discussion: the term "greater digestive power" is confusing.

Re: Thank you for your comment. To clarify the function, the indicated sentence has been modified to: "It is generally accepted that the gallbladder concentrates and stores BAs, thereby enhancing the efficiency of fat digestion and absorption."