# Transcriptomic Analysis Reveals Alterations in Energy Metabolism and Lipogenesis in GIPR/GLP-1R Agonists-Treated Metabolic Dysfunction-associated Steatohepatitis Mice

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## Introduction

Metabolic Dysfunction-Associated Fatty Liver Disease (MAFLD) and its progressive form, Metabolic Dysfunction-Associated Steatohepatitis (MASH), pose a significant global health challenge. The lack of regulatory-approved MASH medications underscores the critical need for innovative treatments. Various gastric inhibitory polypeptide receptor (GIPR) and glucagon-like peptide-1 receptor agonists (GLP-1RAs) are undergoing clinical trials at different stages for MASH, potentially providing new avenues for MASH therapy. Despite previous research reporting reduced liver enzymes and improved MASH histopathology in response to GLP-1RAs, the fundamental molecular mechanism remains obscure. Semaglutide (SEM) and Tirzepatide (TZP) are both under clinical investigation for MASH therapy, with promising therapeutic potential. This study aimed to investigate the differential responses to SEM and TZP at the transcriptional level in MASH mice. Additionally, SEM and TZP both reduce appetite and food intake through their actions in the CNS, which is a significant mechanism in their effect on metabolic syndrome modulation. However, MASH is a unique liver manifestation of metabolic syndrome, characterized by significant hepatic lipid accumulation, inflammatory response, and fibrosis. This study also examined the mechanism of action of SEM and TZP beyond satiety and food consumption regulation by introducing corresponding dietary restriction groups.

## Experimental Design

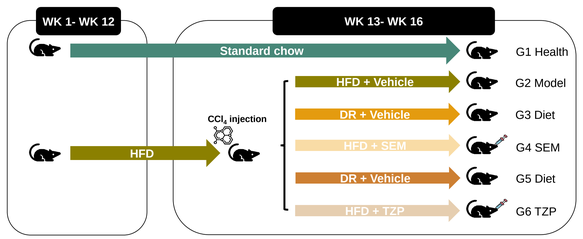


**Figure 1. Generation of MASH mouse model and treatment** The C57BL/6J mice were fed with high-fat diet (HFD) for 12 weeks with $CCl_4$ injection for MASH development. Semaglutide or Tirzepatide was dosed subcutaneously twice a week. Diet restriction (DR) was introduced as relative controls of SEM and TZP.

## Results

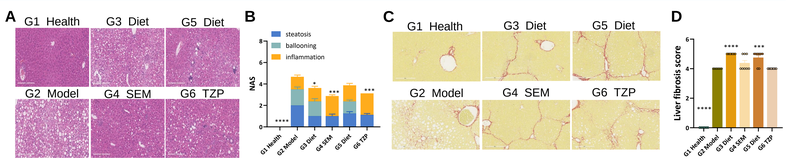


**Figure 2. SEM and TZP alleviates the NAFLD and liver fibrosis** (A) HE staining. (B) NAS score including steatosis, ballooning and inflammation. (C) Sirius Red staining. (D) Liver fibrosis score. Mean ± SEM. *$p$<0.05, ***$p$<0.001, ***$p$<0.0001, compared with Model group.

| Parameters | Healthy | Model | Diet (*DR) | Semaglutide | Diet (#DR) | Tirzepatide |
|---|---|---|---|---|---|---|
| Serum AST (U/L) | 25.66±2.28 | 250.6±18.03 | 62.89±7.09 | 51.19±5.12 | 90.89±11.86 | 62.66±6.57 |
| Serum ALT (U/L) | 70.17±4.46 | 228.2±21.91 | 82.63±5.79 | 103.1±10.71 | 96.75±7.02 | 99±8.75 |
| Serum TC (mmol/L) | 2.56±0.09 | 6.32±0.36 | 3.79±0.21 | 3.25±0.15 | 4.2±0.1 | **3.35±0.21**** |
| Serum TG (mmol/L) | 0.79±0.1 | 1.09±0.08 | 0.78±0.05 | **0.61±0.03**** | 0.77±0.03 | 0.66±0.04 |
| Serum LDL-C (mmol/L) | 0.32±0.02 | 1.3±0.08 | 0.63±0.05 | 0.58±0.06 | 0.73±0.03 | 0.62±0.05 |
| Serum HDL-C (mmol/L) | 2.04±0.06 | 4.11±0.2 | 2.78±0.14 | 2.37±0.09 | 3.04±0.06 | 2.41±0.14 |
| Liver TC (μg/mg) | 2.69±0.18 | 4.2±0.17 | 2.85±0.28 | 3.14±0.23 | 3.27±0.08 | 3.57±0.29 |
| Liver TG (μg/mg) | 28.93±5.54 | 122±4.73 | 23.57±7.15 | 28.56±7.24 | 39.62±5.36 | 41.56±8.52 |
| Liver Weight (D28, mg) | 1195.12±18.92 | 1987.93±128.50 | 1500.53±86.64 | **1086.13±39.30***** | 1472.51±40.49 | **1124.55±32.13***** |

**Table 1. Liver TG (Triglycerides), TC (Total Cholesterol) and serum parameters**
Data are presented as Mean ± SEM. ***$p$ < 0.001, **$p$ < 0.01, *$p$ < 0.05, compared with Diet group.

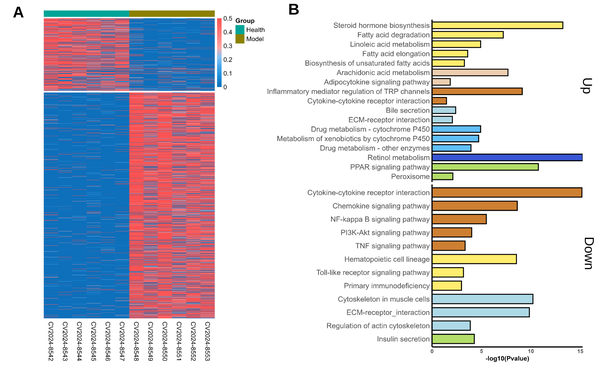


**Figure 3. Alteration of the gene-expression profile in MASH mice** (A) Hierarchical heatmap. (B) KEGG pathway enrichment analysis of the top up-regulated (upper) or down-regulated (lower) pathways.

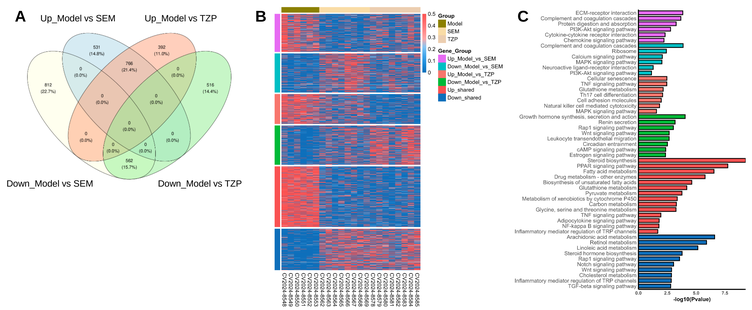


**Figure 4. Comparison of SEM and TZP in MASH treatment: alteration in transcriptional profiles**
(A) Venn diagram of overlap genes in SEM and TZP treatment groups. (B) Hierarchical heatmap. (C) KEGG pathway enrichment analysis of the top up-regulated (upper) or down-regulated (lower) pathways.

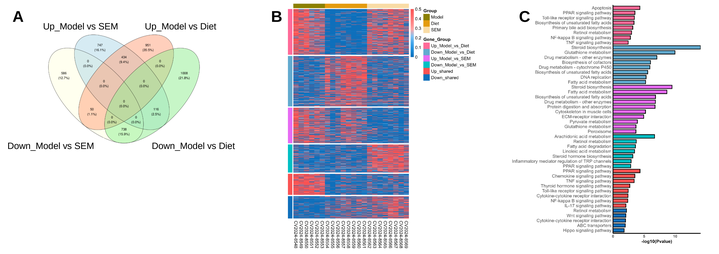


**Figure 5. Comparison of SEM treatment and corresponding dietary restriction in MASH mice: alteration in transcriptional profiles** (A) Venn diagram of overlap genes in SEM and corresponding dietary restriction groups. (B) Hierarchical heatmap. (C) KEGG pathway enrichment analysis of the top up-regulated (upper) or down-regulated (lower) pathways.

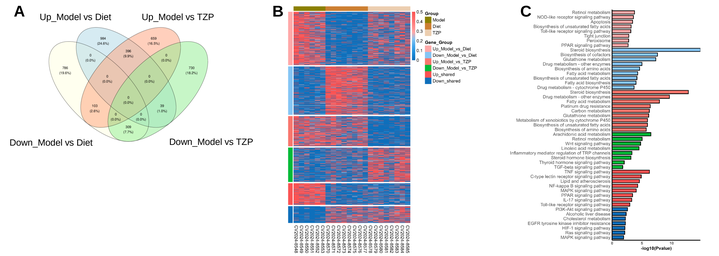


**Figure 6. Comparison of TZP treatment and corresponding dietary restriction in MASH mice: alteration in transcriptional profiles** (A) Venn diagram of overlap genes in TZP and corresponding dietary restriction groups. (B) Hierarchical heatmap. (C) KEGG pathway enrichment analysis of the top up-regulated (upper) or down-regulated (lower) pathways.

## Conclusions

1. Both SEM and TZP treatments yield a significant reduction in the NAS score in MASH mice, but no substantial changes occur in the liver fibrosis score.
2. The HDF+$CCl_4$ MASH model can replicate human MASH histopathology. The transcriptional profiling suggests significant alterations in the pathways of lipid metabolism, xenobiotic metabolism, inflammation, and extracellular matrix regulation. It's noteworthy that the MASH model also presents significant changes in retinol metabolism and PPAR signaling pathways, suggesting their potential roles in MASH pathogenesis.
3. At the transcriptional level, SEM and TZP treatments exhibit a broad overlapping effect in pathways such as steroid biosynthesis, PPAR signaling regulation, and arachidonic acid and retinol metabolism regulations. However, SEM shows distinctive action in the PI3K-Akt pathway and complement and coagulation cascade, whereas TZP shows action in TNF signaling and growth hormone regulation, suggesting divergence in their therapeutic efficacy.
4. Both SEM and TZP show distinctive modulation effects on retinol metabolism, PPAR signaling, and other inflammatory pathways compared to their corresponding dietary restriction controls. This suggests additional beneficial effects beyond food intake regulation for SEM and TZP.

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