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Phosphatidylethanolamine N-methyltransferase (PEMT) Knockout Mice Exhibit Worse Alcohol-Induced Liver Injury than Wildtype Mice

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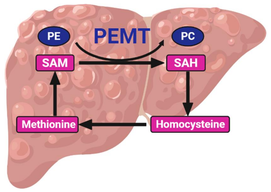
Introduction

The PEMT enzyme catalyzes the successive transfer of 3 methyl groups from S-adenosylmethionine (SAM) to phosphatidylethanolamine (PE) to generate phosphatidylcholine (PC) (1). The PC generated via the PEMT-mediated catalysis is essential for exporting fat out of the liver and thus, is vital for preventing hepatic steatosis (fat accumulation).



Alcohol is known to induce steatosis, and it accomplishes this through various mechanisms including inhibiting PEMT-mediated catalysis via increasing SAH to reduce hepatocellular SAM: S-adenosylhomocysteine (SAH) ratio. Based on these considerations, we were curious about the potential effect of PEMT loss on alcohol-induced liver injury. We hypothesized that the absence of the PEMT enzyme would worsen indices of alcohol-induced liver injury.

Materials and Methods

Animal Handling and Diet

Male PEMT -/- (KO) and wild-type (WT) mice were obtained from Jackson Laboratories. These mice were subjected to chronic + binge alcohol treatment (2). WT and KO mice were fed either the Lieber-DeCarli control or ethanol diets for 10 days, and gavaged on the following day with either saline or ethanol, respectively. Nine hours later the mice were sacrificed, and the blood and liver were collected for the following analyses:

Triglyceride Quantification

Lipids were extracted by Folch method and triglyceride levels were quantified using the Thermo DMA Kit (3).

SAM SAH Analysis

Liver tissue was sonicated in 0.5N perchloric acid and the supernatant was analyzed by HPLC.

ALT Levels

Serum ALT was analyzed using a VITROS 5.1 FS Chemistry System (4).

Hepatic Histology

A portion of the liver was fixed in 10% neutral-buffered formalin. Paraffin sections were prepared and stained with hematoxylin and eosin, followed by imaging using a Keyence BZ-810 microscope.

Results

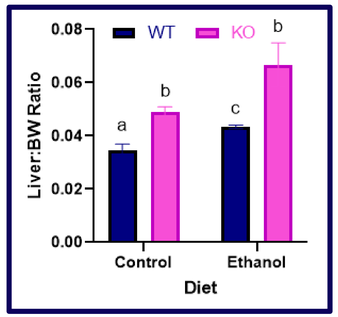


Figure 1: Ethanol-fed PEMT KO mice have higher liver to body weight (BW) ratios

Ethanol-fed KO mice liver:BW ratio was 36.7% and 55.8% higher than control-fed KO and ethanol-fed WT mice, respectively. Data is presented as the average ± SEM for each treatment group. Values not sharing a common subscript letter are statistically different, $p < 0.05$.

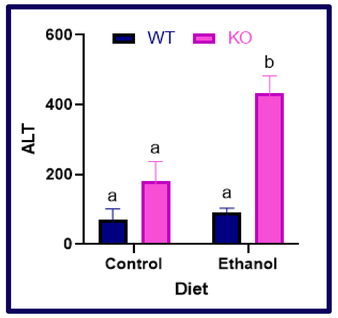


Figure 2: Ethanol-fed PEMT KO mice exhibit worse alcohol-induced liver damage

PEMT KO mice on ethanol diet showed drastically increased serum ALT levels compared against all groups. Data is presented as the average ± SEM for each treatment group. Values not sharing a common subscript letter are statistically different, $p < 0.05$.

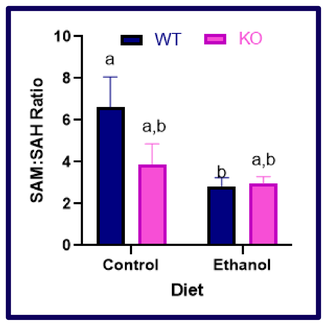


Figure 3: Intracellular SAM:SAH ratios in WT and PEMT KO mice fed control or alcohol diet

Ethanol-fed KO mice had reduced SAM:SAH ratios compared to both control groups, however, the change was not significant. Data is presented as the average ± SEM for each treatment group. Values not sharing a common subscript letter are statistically different, $p < 0.05$.

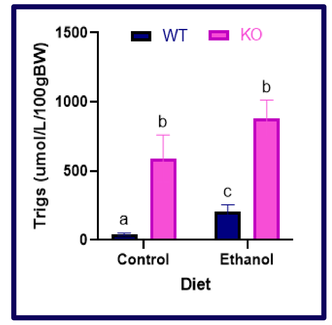


Figure 4: Ethanol-fed PEMT KO mice show increased triglyceride levels

Triglyceride levels in ethanol-fed KO mice were 322% greater than WT ethanol and 49.6% greater than KO control. Data is presented as the average ± SEM for each treatment group. Values not sharing a common subscript letter are statistically different, $p < 0.05$.

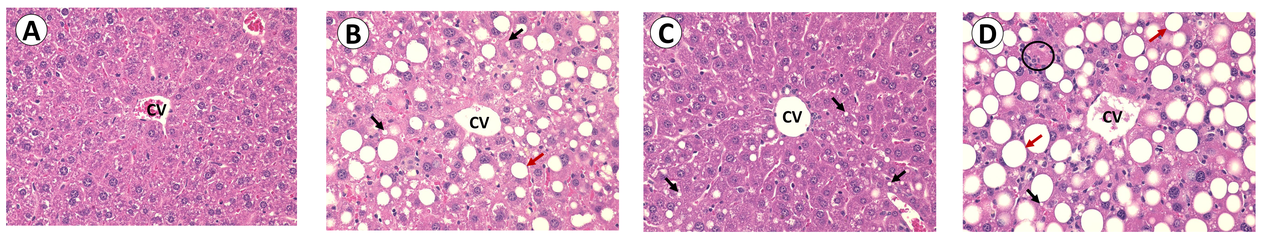


Figure 5: Steatosis in PEMT KO mice is exacerbated by alcohol administration

(A) WT control; (B) KO control; (C) WT ethanol; (D) KO ethanol (H&E Staining; magnification 40X). Both the control and ethanol-fed KO mice exhibited significantly increased macrovesicular and microvesicular steatosis, with the ethanol-fed KO mice showing overall worse liver-injury. The black arrowheads identify microvesicular steatosis, while the red shows macrovesicular; CV identifies the central vein. The black circle identifies inflammation.

Summary

1. Ethanol-fed PEMT KO mice display increased liver to body weight ratios, as well as triglyceride levels.
2. ALT levels were drastically elevated in ethanol-fed PEMT KO and were significantly increased compared with the other treatment groups.
3. SAM:SAH ratio is reduced in ethanol fed WT compared to its respective control. Both control and ethanol fed KOs had numerically reduced SAM:SAH ratio compared with WT control.
4. Ethanol-fed PEMT KO mice exhibit increased macrovesicular and microvesicular steatosis.

Discussion

Previous findings have demonstrated that removing PEMT activity results in liver steatosis. Additionally, it is well-known that alcohol consumption induces liver damage. Our study reveals that there is additional liver damage when these two are combined.

Overall Conclusions

1. PEMT KO mice exhibit similar liver damage to what is seen in ethanol-fed WT.
2. PEMT KO mice fed ethanol exhibit worse liver injury compared to ethanol-fed WT or both non-alcohol-consuming PEMT KO and WT mice.
3. Similar results are also seen in female mice.
4. Lack of PEMT increases susceptibility to alcohol-induced liver damage, and this mechanism is a field for future study.

Acknowledgments

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References

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