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Phosphatidylethanolamine Methyltransferase Deficiency Exacerbates Acute Alcohol-Induced Liver Injury

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Introduction

Alcohol-associated liver disease (ALD) is a global burden of healthcare and remains a major cause of morbidity and mortality worldwide. ALD includes a spectrum of injuries that progresses from hepatic steatosis, alcoholic hepatitis to alcohol-associated cirrhosis and even hepatocellular carcinoma with continued alcohol misuse. The development of ALD depends on several factors, including genetics. The liver enzyme phosphatidylethanolamine methyltransferase (*PEMT*) catalyzes three sequential methyl transfers to phosphatidylethanolamine, generating phosphatidylcholine (PC). The PC generated with PEMT-mediated catalysis is preferentially used in very-low-density-lipoprotein (VLDL) assembly and is required for its normal biogenesis and secretion (1-3). Alcohol affects the methylation potential and impairs *PEMT* activity, which by inhibiting VLDL synthesis contributes to the development of hepatic steatosis (4). Polymorphisms in the human *PEMT* gene causing loss of function confer susceptibility to metabolic-associated steatohepatitis (5).

Aim

Based on these considerations, we hypothesized that *PEMT* deletion would exacerbate alcohol induced liver injury.

Methods

Animal Handling and Diet:
Male and female wildtype (WT) and *PEMT* knock out (KO) mice (12 weeks of age) were subjected to ethanol binge feeding model. The animals were gavaged with maltose dextrin or ethanol (5g/Kg BW) twice, 12 hours apart. The mice were euthanized eight hours after the second dose, where the blood and liver were collected for the following analyses:

AST and ALT levels: Serum AST and ALT were analyzed using a VITROS 5.1 FS Chemistry System.

Hepatic histopathology: Neutral-buffered formalin fixed liver sections stained with hematoxylin & eosin (H & E) and picrosirius red were imaged using a Keyence BZ-810 microscope.

HPLC Analysis: Liver tissues were homogenized in 0.5N perchloric acid and subjected to HPLC analysis to determine S-adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH) levels (3,4) The SAM:SAH ratio, or methylation index, was calculated, as detailed (3,4).

Triglyceride Quantification: Lipids were extracted by Folch method (6) and triglyceride levels were quantified using the Thermo DNA Kit (3,4).

Enzymatic Activity: Lysosomal acid lipase and proteasome activities were determined in liver homogenates, as detailed (7)

Statistical Analyses: Data are expressed as mean values ± standard error (SE). Values not sharing a common subscript letter are statistically different, $p < 0.05$.

References

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(4) Kharbanda et al. *J Nutr* 2005; 135: 519-524.
(5) Song et al. *FASEB J* 2005; 19: 1266-1271.
(6) Folch et al. *J Biol Chem* 1957; 226: 497-509
(7) Kharbanda et al. *Exp Mol Pathol* 2014; 97: 49-56

Results

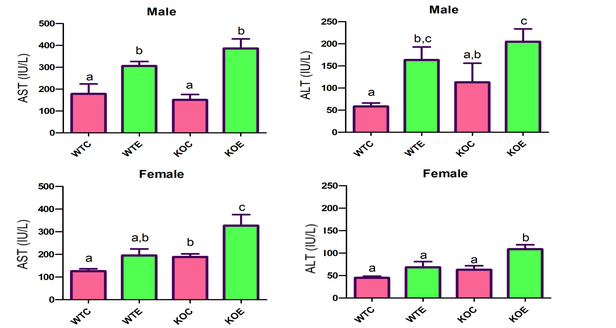
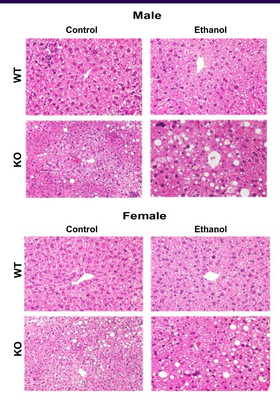


Figure 1: Serum AST and ALT levels increased with ethanol consumption, with the highest levels shown in ethanol-fed *PEMT* KO mice, indicating liver damage. **Figure 2:** Histopathological assessment (H&E Staining, magnification 40X) showing ethanol exposure increases hepatic steatosis, wich is more pronounced in the KO mice.



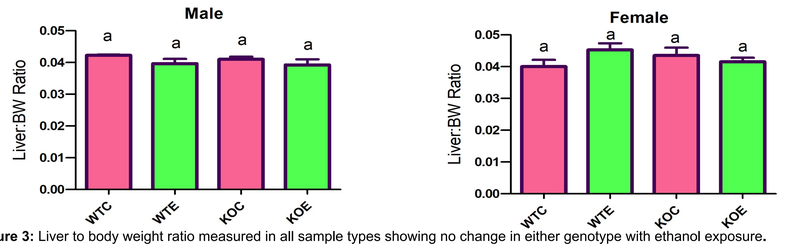


Figure 3: Liver to body weight ratio measured in all sample types showing no change in either genotype with ethanol exposure.

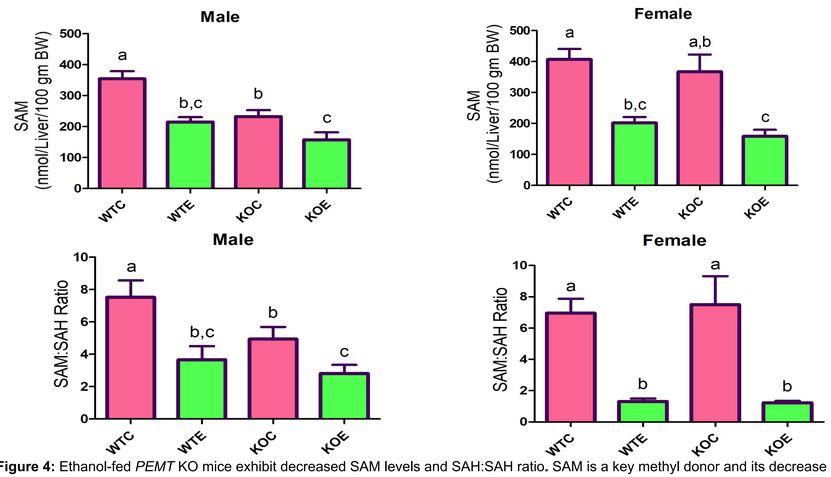


Figure 4: Ethanol-fed *PEMT* KO mice exhibit decreased SAM levels and SAH:SAH ratio. SAM is a key methyl donor and its decrease indicates a reduced methylation potential in *PEMT* KO mice, increasing the risk of alcohol-induced liver injury.

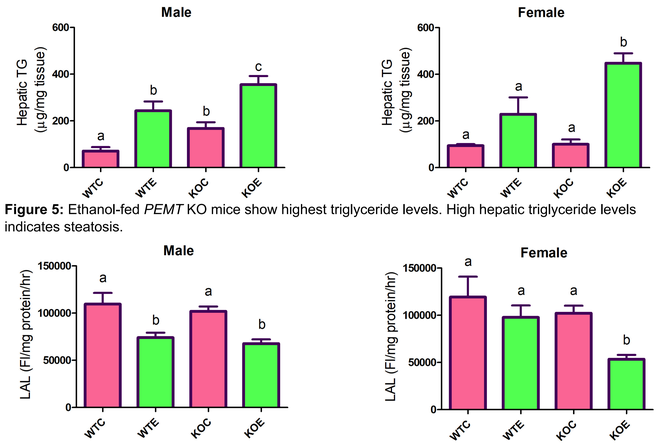


Figure 5: Ethanol-fed *PEMT* KO mice show highest triglyceride levels. High hepatic triglyceride levels indicates steatosis.

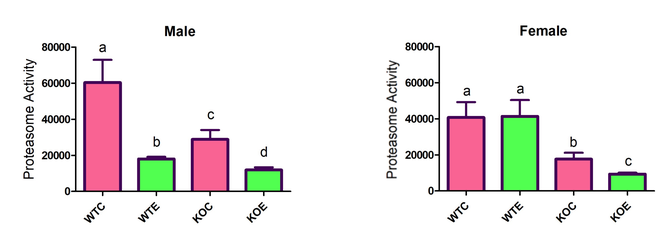


Figure 8: Lysosomal acid lipase and proteasome activity is reduced the most in ethanol-fed *PEMT* KO mice. Low LAL activity interferes with the hydrolysis of triglycerides. Low proteasome activity suggests increased accumulation of damaged proteins in the KO mice.

Conclusion

✓Deletion of *PEMT* exacerbates acute alcohol-induced liver injury in both males and females as evidenced by:

- Increased AST and ALT levels
- Increased fat accumulation by histopathological assessment
- Decreased SAM levels causing a reduction in the methylation potential
- Increased hepatic triglycerides
- Decreased lysosomal acid lipase activity
- Decreased proteasome activity

Acknowledgements

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