The Effects of Age and Alcohol on Lipid Metabolism in the Liver

Jessica S. Yoon[1,3], Sundararajan Mahalingam[1,3], Jacy L. Kubik[1,3], Ramesh Bellamkonda[1,3], Carol A. Casey[1,2,3], Kusum K. Kharbanda[1,2,3], and Karuna Rasineni[1,2,3]

1. Departments of Internal Medicine and 2. Biochemistry & Molecular Biology, University of Nebraska Medical Center, Omaha, NE.
3. Research Service, Veterans' Affairs Nebraska-Western Iowa Health Care System, Omaha, NE.

UNMC UNIVERSITY OF NEBRASKA MEDICAL CENTER

Summer Undergraduate Research Program

Introduction

Alcohol misuse is an ongoing prevalent issue in the US and worldwide. Over 90% of individuals with excessive alcohol use develop alcohol-associated steatosis, characterized by lipid accumulation in hepatocytes.[1] Most patients with serious alcohol-associated liver disease (ALD) generally manifest signs and symptoms in the 4th and 5th decades of life to suggest that aging is an important contributor to liver disease progression. Age is also a predictor for ALD-related mortality.[2] There is increasingly greater concern for age as a predictor for ALD, as data trends predict that more than 20% of the population will be 65+ by the year 2029.[3] Furthermore, this older population had the highest average annual increases in binge drinking from 2000-2015 among all age groups.[4] Thus, the goal of this study was to examine how aging and alcohol affect lipid metabolism in the liver.

Methods

Animals and diet: Male Wistar rats of ages 4 months, 8 months, 12 months and 22 months-old were pair-fed Lieber-DeCarli control or ethanol diet. After 6 weeks of feeding, the rats were euthanized. Blood was collected and the livers were excised and stored at -70ºC until used for analyses.
Histological analysis: Five µm thick formalin tissue sections were stained with H&E staining for observing pathological assessment.
Non-esterified free fatty acids (NEFA): Serum NEFA were quantified using the NEFA-HR (2) diagnostic kit from Wako Life Sciences (Mountain View, CA).
Hepatic triglycerides: Hepatic triglycerides were extracted using the Folch method. Lipid extract was quantified by using a diagnostic kit (#TR22421 from Thermo Fischer Scientific).
Gene expression analysis: RNA was isolated from tissue samples using the PureLink RNA Mini Kit (Invitrogen, Carlsbad, CA) and was reverse transcribed from 1 µg of total RNA using Taqman Reverse Transcription Reagents and SYBR Green Reverse Transcription Reagents (Applied Biosystems). Quantitative PCR (qPCR) was performed using rat-specific Taqman and SYBR Green primers.
Statistical analysis: Data were analyzed by t-test. Values not sharing the same letter are significantly different.

Results

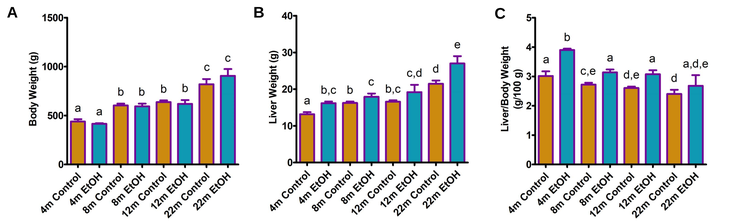

Figure 1. General observations of 4 months, 8 months, 12 months and 22 months-old rats pair-fed the control or ethanol diet. A) Body weight. B) Liver weight. C) Ratio of liver to body weight. Values are mean±SEM, n = 8.

Results

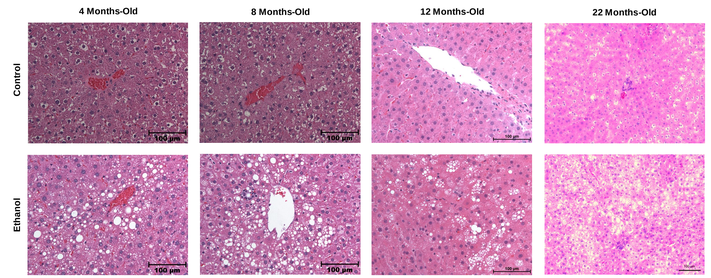

Figure 2. Hematoxylin and eosin-stained sections of representative livers of 4 months, 8 months, 12 months, and 22 months-old rats.

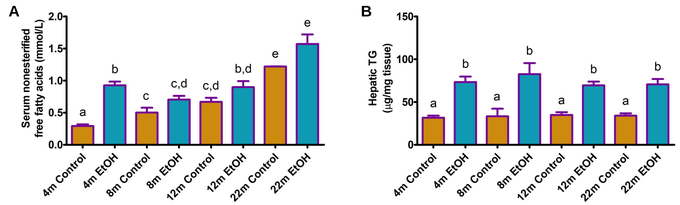

Figure 3. A) Serum NEFA levels in rats. B) Quantitative analysis of hepatic triglyceride content. Values are mean±SEM, n = 8.

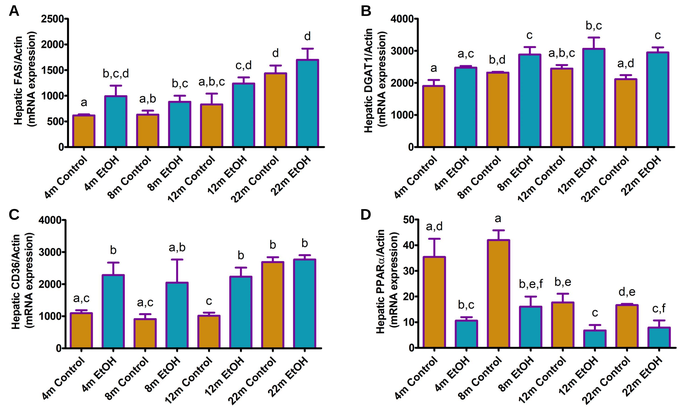

Figure 4. Hepatic gene expression of A) fatty acid synthase (FAS), B) diacylglycerol O-acyltransferase 1 (DGAT1), C) CD36, and D) peroxisome proliferator-activated receptor alpha (PPARα). Values are mean±SEM, n = 8.

Summary

1. Both body weight and liver weight increased with advancing age. Chronic ethanol feeding increased liver to body weight ratio across all age groups.
2. Histopathological evaluation of hematoxylin and eosin-stained liver sections showed steatosis in ethanol-fed rats across all age groups.
3. Quantitative analysis demonstrated an increase of serum free fatty acids in ethanol-fed rats across all age groups. There was also an overall increase in serum free fatty acids with advancing age. Furthermore, chronic ethanol feeding significantly increased liver triglyceride levels across all age groups of rats.
4. Chronic ethanol feeding increased fatty acid and triglyceride esterification, represented by FAS and DGAT1 expression, respectively, across all age groups. Furthermore, FAS was increased as age increased. Ethanol feeding increased fatty acid transport/uptake, as indicated by increased CD36 expression, across all age groups except the 22 months-old rats. However, both the control and ethanol-fed 22 months-old rats exhibited increased fatty acid transport CD36, which correlates with an increase of circulating free fatty acids in these rats. Ethanol feeding also induced a decrease in PPARα expression. Age intensified this decrease in expression.

Conclusion

These results demonstrate the effect of alcohol on lipid metabolism in the liver and indicate that age can exacerbate the effects of alcohol-associated liver disease.

Acknowledgements

This work was supported by the NIH R01AA028504 grant and the UNMC SUARP.

References

1. Patel, R., & Mueller, M. (2022). Alcoholic Liver Disease. In *StatPearls*. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/books/NBK546632/
2. Masson S, Emmerson I, Henderson E, Fletcher EH, Burt AD, Day CP, et al. Clinical but not histological factors predict long-term prognosis in patients with histologically advanced non-decompensated alcoholic liver disease. *Liver Int.* 2014;*34*(2):235-42.
3. El Hayek, S., Geagea, L., El Bourji, H., Kadi, T., & Talih, F. (2022). Prevention Strategies of Alcohol and Substance Use Disorders in Older Adults. *Clinics in Geriatric Medicine*, *38*(1), 169–179. https://doi.org/10.1016/j.cger.2021.07.011
4. Grucza, R. A., Sher, K. J., Kerr, W. C., Krauss, M. J., Lui, C. K., McDowell, Y. E., Hartz, S., Virdi, G., & Bierut, L. J. (2018). Trends in Adult Alcohol Use and Binge Drinking in the Early 21st Century United States: A Meta-Analysis of Six National Survey Series. *Alcoholism, Clinical and Experimental Research*, *42*(10), 1939–1950. https://doi.org/10.1111/acer.13859