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Ethanol and Nutrient Stressors Synergistically Increase Lipid Content in Hepatocyte Spheroids While Differentially Dysregulating Hepatocyte Lipid Metabolism

Introduction: In the United States, approximately one-third of the adult population has steatotic liver disease (SLD). Metabolic dysfunction, alcohol misuse, and the combination are drivers of MASLD, ALD, and MetALD, respectively. There is currently only one FDA-approved treatment for MASLD and only lifestyle intervention for ALD management. It remains unclear how the simultaneous presence of alcohol and metabolic stressors mechanistically contribute to liver injury. The present study uses a 3D human hepatocyte model of metabolic and alcohol-associated stress to identify mechanisms of lipid metabolism and storage dysregulation due to the combined effects of high alcohol, sugars, and fats.

Methods: HepaRG spheroids were formed at 2,000 cells per well over 3-4 days. Spheroids were then switched to one of four experimental media: control (5.5 mM glucose and vehicle (C)); ethanol (50 mM (E10)); high sugar (11 mM glucose and 11mM fructose) and high fat (300 µM oleic acid and 300 µM palmitic acid (SF10)); or ethanol, high sugar, and high fat (ESF10) media for 10 days. Spheroids were collected at endpoint for triglyceride quantification, ATP quantification, trypan blue staining, and RT-qPCR. Gene targets used were related to lipid metabolism regulation and results were analyzed using two-way ANOVA with Fisher's exact test for post-hoc analyses. The alpha value was set to 0.05 to indicate statistical significance.

Results: Ethanol increased mRNA expression of the alcohol-metabolizing enzyme cytochrome P450 2E1 ($p=0.0189$). In addition, ESF10 increased triglyceride content compared to all other groups ($p<0.0001$). Ethanol had a main effect of increasing mRNA expression of the lipid synthesis enzymes acetyl-CoA carboxylase ($p=0.0463$) and fatty acid synthase (FASN; $p=0.0011$). Ethanol also increased gene expression of the lipid regulators peroxisome proliferator-activated receptor alpha ($p=0.0498$) and carbohydrate responsive element binding protein ($p=0.0042$). Sugar fat treatment had a main effect of decreasing FASN expression ($p=0.0103$). Ethanol and sugar fat had main effects of increasing expression of lipid storage protein perilipin 1 ($p=0.0493$ and $p=0.0391$, respectively). Carnitine palmitoyltransferase I, an enzyme important in beta-oxidation, expression showed an interaction between ethanol and sugar fat ($p=0.0166$), with increases in expression in ESF10 compared to all other groups ($p<0.01$). Ethanol along with sugar fat treatments had main effects of decreasing ATP content in HepaRG spheroids (both $p<0.0001$), but only E10 and ESF10 showed qualitatively notable increases in trypan blue dye uptake in peripheral cells of the spheroids.

Conclusion: Decreases in ATP results suggest that ethanol, high sugar, and high fats reduce the bioenergetic capacity of HepaRG cells. Future studies will explore whether this is due to impaired mitochondrial function or altered metabolic profiles. Gene expression results showed increased expression of lipogenesis-related genes with ethanol treatment, whereas only the ESF10 group showed an increase in triglyceride concentration. Further research that separates sugars and fats will help clarify the synergistic effects of alcohol and metabolic stressors on hepatocyte lipid dysregulation.