Primary Rat Hepatocyte Spheroids as a Model of MetALD

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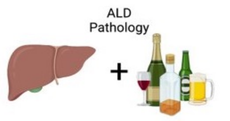
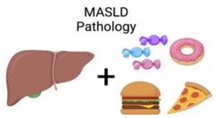
NIH National Institute on Alcohol Abuse and Alcoholism

Introduction

- MetALD encompasses both alcohol-associated liver disease (ALD) and metabolic dysfunction-associated steatotic liver disease (MASLD)
- *ALD is due to alcohol consumption*
- *High fat and sugar diet contributes to MASLD*





Cellular Determinants:

- **Metabolic Stress**
- Oxidative Stress/Mitochondrial Damage
- **Inflammation**
- Fibrosis

- Alcohol and metabolic liver injury as well as LPS from subsequent gut-leak activate the TLR-4 pathway leading to IL-6, IL-1B, and CRP production

Objective & Hypothesis

The objective of this study was to model the metabolic and inflammatory response of primary rat hepatocyte spheroids treated with high fats and sugars (HFS) plus ethanol (E) after chronic control or alcohol-diet feeding.

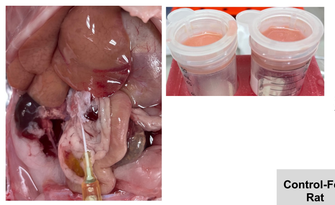
Hypothesis

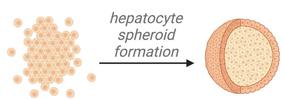
Primary rat hepatocyte spheroids treated with HFS + E will have greater lipid accumulation and a larger immune response than control spheroids or those treated with HFS or E alone.

Methods

Isolate primary rat hepatocytes | **Culture spheroids** | **Bioluminescent assays, ELISA, & RT-qPCR**

Two-step liver perfusion & collagenase digestion
Percoll & centrifugation hepatocyte isolation

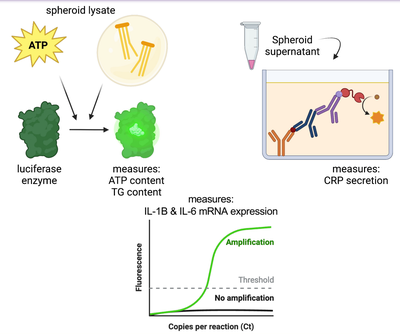




Spheroid Culture Conditions (Days 5-10):

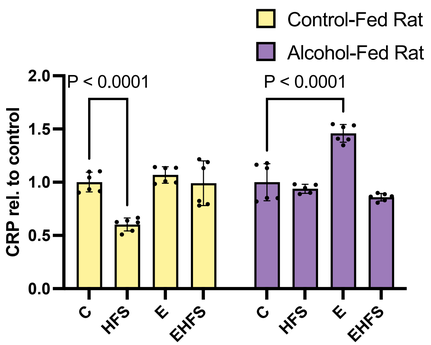
	Control Media	50mM EtOH Media	HFS Media*	EtOH + HFS Media
Control-Fed Rat	C-C	C-E	C-HFS	C-EHFS
Alcohol-Fed Rat	A-C	A-E	A-HFS	A-EHFS

Day 10:
+/- 6hr
100ng/mL
LPS
stimulation

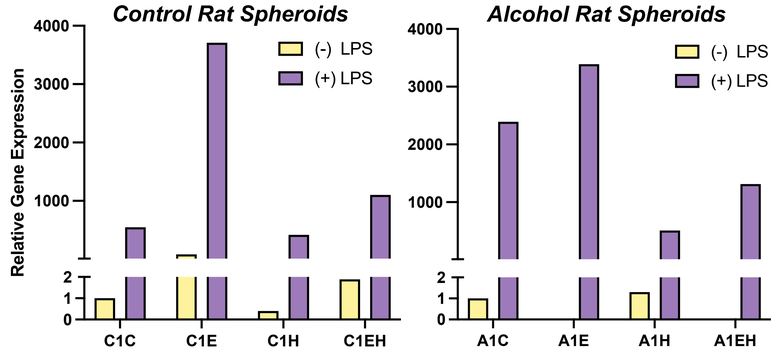


Results

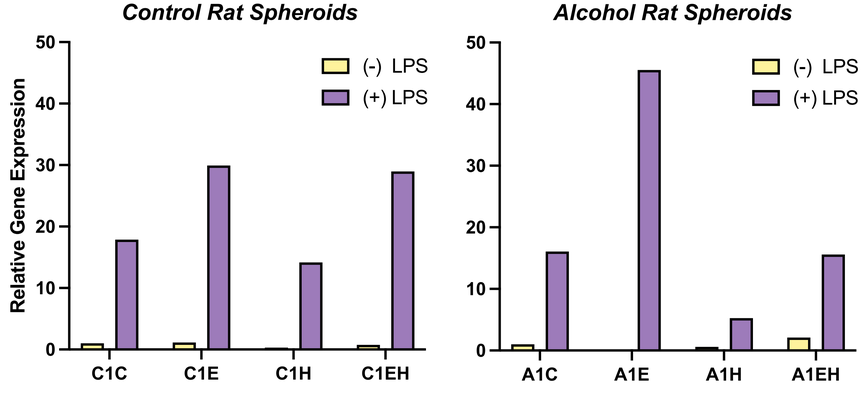
CRP secretion



IL-1B gene expression

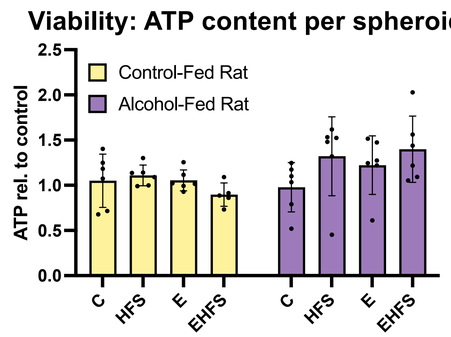


IL-6 gene expression

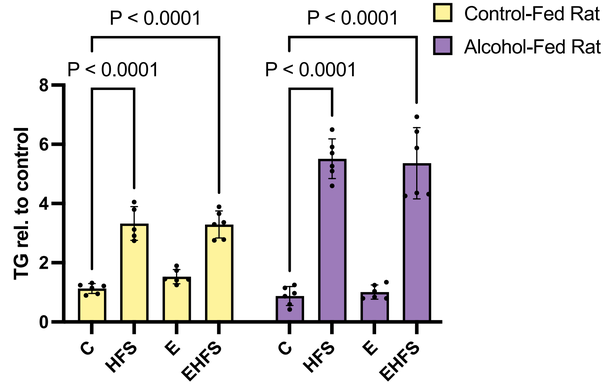


Results

Viability: ATP content per spheroid



Modeling MetALD: Triglyceride content per spheroid



Discussion

- All spheroids showed similar viability as indicated by ATP content.
- TG accumulation was significantly increased by in vitro HFS and EHFS in both control and alcohol-fed rat spheroids.
- CRP secretion was highest after ethanol treatment in alcohol-fed rat spheroids.
- Pro-inflammatory cytokine expression was elevated by LPS stimulation and IL-1B was increased by ethanol treatment in control-rat spheroids.

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Images created using Biorender

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