Ethanol, sugar, and fat synergistically dysregulate hepatocyte lipid metabolism

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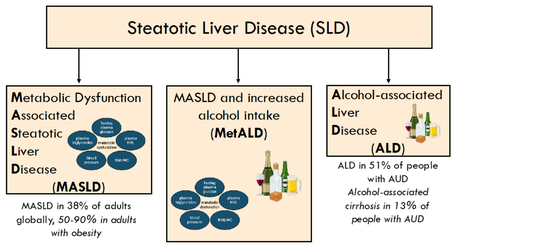
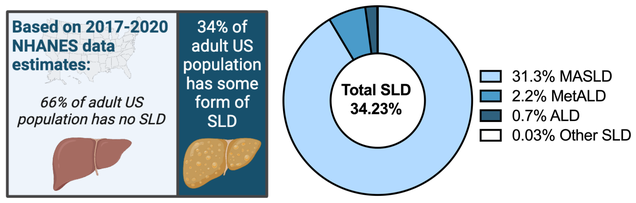
Introduction

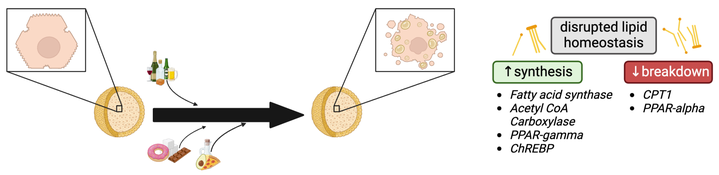
- In the United States, approximately one-third of the adult population has steatotic liver disease (SLD), which is significant fat accumulation in the liver. Metabolic dysregulation as well as excess alcohol consumption are two risk factors for developing SLD.





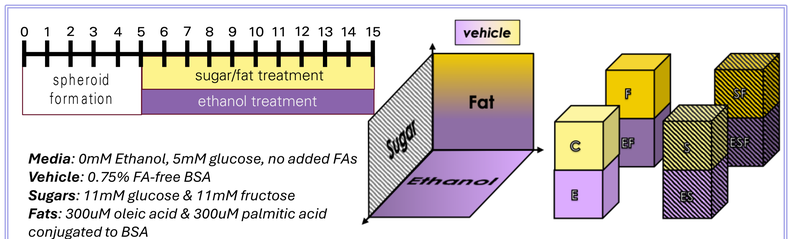
- Only one FDA-approved MASLD treatment exists while MetALD/ALD relies on lifestyle management alone.
- The present study uses a 3D human hepatocyte model to simulate alcohol and metabolic stress to identify causes of lipid dysregulation due to the combined effects of alcohol, high sugars, and high fats.

Hypothesis



- Ethanol, high fats, and high sugars increase lipid accumulation in hepatocyte spheroids via dysregulated lipid storage and breakdown and alterations in sugar and lipid metabolism.

Methods



Media: *0mM Ethanol, 5mM glucose, no added FAs*
Vehicle: *0.75% FA-free BSA*
Sugars: *11mM glucose & 11mM fructose*
Fats: *300uM oleic acid & 300uM palmitic acid conjugated to BSA*

- At endpoint, spheroids were collected for triglyceride and ATP quantification, RNA isolation, and trypan blue dye staining.
- Collected RNA was used for RT-qPCR primarily using genes involved in lipid metabolism regulation.
- Results were analyzed using three-way ANOVA with Tukey's (n>5) or Fisher's LSD test (n<5) for post-hoc analyses (α=0.05).

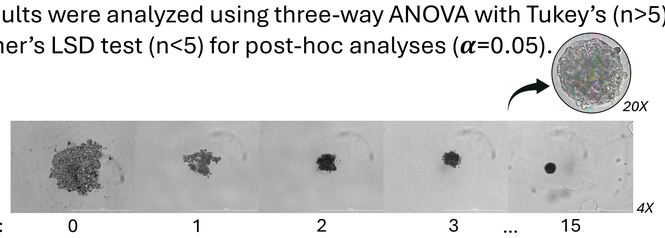
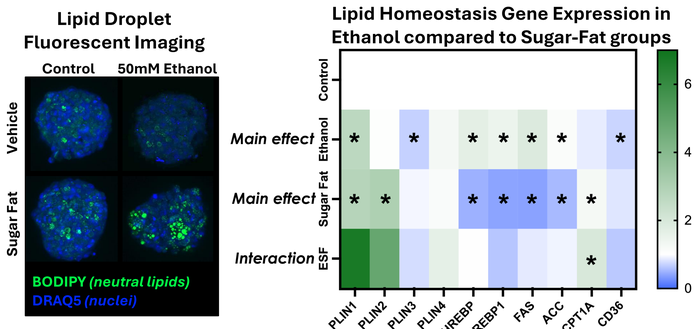


Figure 1. Brightfield images from a single spheroid over time. Images are taken at 4X or 20X as indicated to monitor spheroid formation and size. Imaged using a Cytation 1.

Lipid Quantification



Two-way ANOVA with main effects of ethanol, main effects of sugar fat, and interactions indicated; post-hoc results not shown.

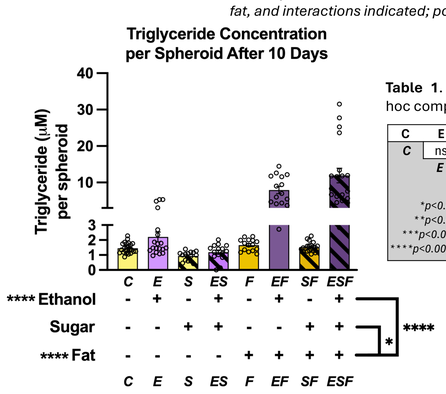


Table 1. Triglyceride concentration post-hoc comparisons.

	C	E	S	ES	F	EF	SF	ESF
C		ns	ns	ns	ns	****	ns	****
E			ns	ns	ns	***	ns	****
S				ns	ns	****	ns	****
ES					ns	****	ns	****
F						****	ns	****
EF							****	*
SF								****

*p<0.05 **p<0.01 ***p<0.001 ****p<0.0001

Figure 2. Triglyceride content. Each dot represents one spheroid. Main effects are shown on the bottom left; interactions are shown on the bottom right. Three-way ANOVA with Tukey's post-hoc test was used.

ATP Quantification

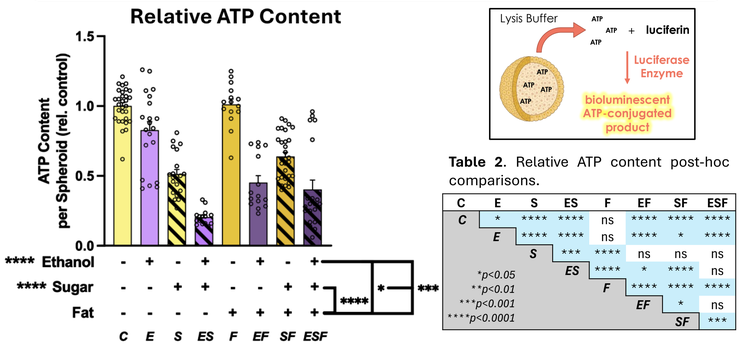


Table 2. Relative ATP content post-hoc comparisons.

	C	E	S	ES	F	EF	SF	ESF
C		*	****	****	ns	****	****	****
E			****	****	ns	****	*	****
S				***	****	ns	ns	ns
ES					****	*	****	ns
F						****	****	****
EF							*	ns
SF								***

*p<0.05 **p<0.01 ***p<0.001 ****p<0.0001

Figure 3. Relative ATP content. Each dot represents one spheroid. Main effects are shown on the bottom left; interactions are shown on the bottom right. Three-way ANOVA with Tukey's post-hoc test was used.

Ethanol drives upregulation of pyruvate kinase in the absence of fat, whereas fat and ethanol in combination upregulate lipid breakdown enzymes

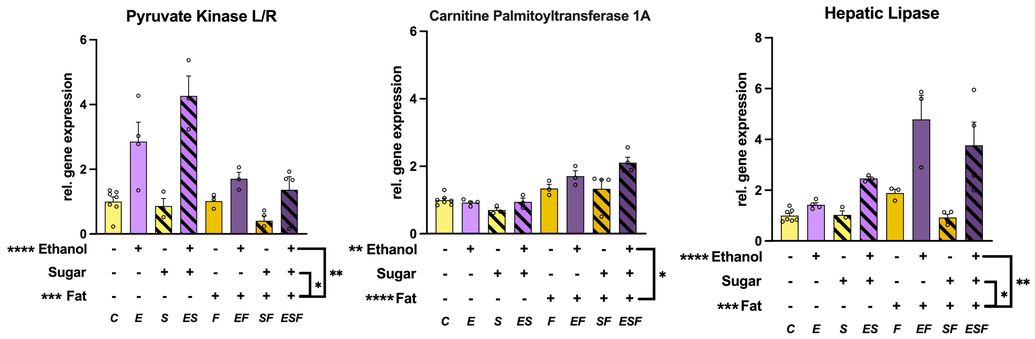


Table 3. Pyruvate kinase relative gene expression post-hoc comparisons.

	C	E	S	ES	F	EF	SF	ESF
C		***	ns	****	ns	ns	ns	ns
E			***	*	**	*	****	**
S				****	ns	ns	ns	ns
ES					****	***	****	****
F						ns	ns	ns
EF							*	ns
SF								ns

*p<0.05 **p<0.01 ***p<0.001 ****p<0.0001

Table 4. CPT1A relative gene expression post-hoc comparisons.

	C	E	S	ES	F	EF	SF	ESF
C		ns	ns	ns	ns	**	ns	****
E			ns	ns	ns	**	*	****
S				ns	ns	**	**	****
ES					ns	**	ns	****
F						ns	ns	**
EF							ns	ns
SF								***

*p<0.05 **p<0.01 ***p<0.001 ****p<0.0001

Table 5. Hepatic lipase gene expression post-hoc comparisons.

	C	E	S	ES	F	EF	SF	ESF
C		ns	ns	*	ns	****	ns	****
E			ns	ns	ns	****	ns	***
S				*	ns	****	ns	***
ES					ns	**	*	ns
F						***	ns	**
EF							****	ns
SF								****

*p<0.05 **p<0.01 ***p<0.001 ****p<0.0001

Figure 4. Rate-limiting glycolytic (pyruvate kinase), fatty-acid oxidation (carnitine palmitoyltransferase 1A; CPT1A), and lipolysis (hepatic lipase) enzymes gene expression. Each dot represents RNA from 20-25 spheroids. Main effects are shown on the bottom left; interactions are shown on the bottom right. Three-way ANOVA with Fisher's LSD post-hoc test was used.

Ethanol and Fat drive upregulation of lipid storage proteins

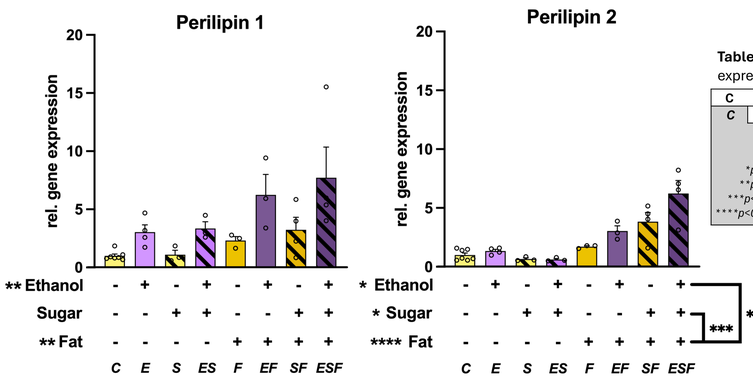


Table 6. Perilipin 2 relative gene expression post-hoc comparisons.

	C	E	S	ES	F	EF	SF	ESF
C		ns	ns	ns	ns	**	***	****
E			ns	ns	ns	*	**	****
S				ns	ns	**	***	****
ES					ns	**	***	****
F						ns	*	****
EF							****	ns
SF								**

*p<0.05 **p<0.01 ***p<0.001 ****p<0.0001

Figure 5. Perilipin gene expression. Each dot represents RNA from 20-25 spheroids. Main effects are shown on the bottom left; interactions are shown on the bottom right. Three-way ANOVA with Fisher's LSD post-hoc test was used if an interaction was present.

Conclusions & Future Directions

- In our **MetALD** hepatocyte injury model, **triglyceride** content was increased by **the combination of ethanol and fat**.
  - *Gene expression results suggest upregulation of lipid storage proteins by ethanol and sugar-fat alone, yet these early changes did not yet lead to a phenotypic response in our model. Additionally, ethanol upregulated lipid synthesis genes whereas sugar-fat decreased these genes.*
- **Decreases in ATP** quantity due to **sugar**, **ethanol**, and **combinations** of **ethanol, sugar, and fat** may suggest that these factors **reduce the bioenergetic capacity of HepaRG cells**.
  - *Future studies will explore whether this is due to impaired mitochondrial function or changes in glycolysis by measuring oxygen consumption rates.*
- Ongoing research will determine the regulation of more genes involved in lipid metabolism and lipid storage, as well as genes involving **mitochondrial and oxidative stress regulation**.
  - In addition, we will determine whether **increasing fatty-acid oxidation using lipid nanoparticle mediated RNA delivery** ameliorates the alterations in ATP and triglycerides due to ethanol, sugars, and fats.

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