Genetically-defined Metabolic Response to Estradiol Stimulation using iPSC-derived Liver Organoids

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Introduction: Interactions between single nucleotide polymorphisms (SNP) and sex hormone pathways involved in the onset and progression of metabolic dysfunction-associated steatotic liver disease (MASLD) have previously been reported. One SNP that is involved in MASLD, GCKR rs1260326:C>T, has varying effects on MASLD pathogenesis in a metabolism-dependent manner. Given that sex-specific differences in metabolism are largely mediated by estrogen receptor-α, we hypothesized that hepatic dysfunction due to the GCKR risk allele may be influenced by estradiol stimulation. Therefore, in this study, we investigated the interaction between hepatic metabolism, the GCKR risk allele, and estradiol stimulation using a human liver organoid (HLO) model derived from isogenic induced pluripotent cell (iPSC) lines.

Methods: An iPSC line with GCKR rs1260326:C/C genotype was gene-edited to create isogenic cell lines with non-risk CC and risk TT genotypes. iPSCs were differentiated into posterior foregut cells and then into HLOs using the protocol outlined by Shinozawa 2021. Two assays were preformed using mature HLOs around day 30: an estradiol-17β (E2) dose dependency study (0, 1, 10 nM) and a lipid loading study (baseline, 300 uM oleic acid, 1000 nM E2, and oleic acid + E2). After 48 hours of stimulation, HLOs were lysed for RNA extraction. Extracted RNA was then used to generate cDNA. Quantitative PCR on generated cDNA was used to assess expression of fatty acid oxidation and lipogenesis targets. Live cell staining was used to qualitatively assess lipid accumulation in the lipid loading study.

Results: In the E2 dose-dependency study, several fatty acid oxidation (PPARα, CPT1a) and lipogenesis (SREBF1c, FASN) genes increased proportional to E2 stimulation. In the lipid loading study, CC organoids generally expressed higher levels of fatty acid oxidation and lipogenesis targets than TT organoids. Furthermore, E2 exposure primed GCKR CC, but not TT, organoids to increased expression of fatty acid oxidation and lipogenesis genes. Furthermore, CC organoids displayed a reactive change in lipid accumulation upon E2 stimulation, whereas TT organoids lacked a similar response to E2 stimulation.

Conclusion: While estradiol proportionally increases both fatty acid anabolic and catabolic pathways in the non-risk condition, the lipid loading study suggests that GCKR TT reduced the plasticity of the liver metabolic response to estradiol stimulation when compared to GCKR CC. These results indicate the complex interplay among estradiol stimulation, genetics, and hepatic metabolism using iPSC-derived HLOs. Further studies are needed to explore the molecular mechanisms by which estradiol-induced metabolic responses can be modified by GCKR genotype.

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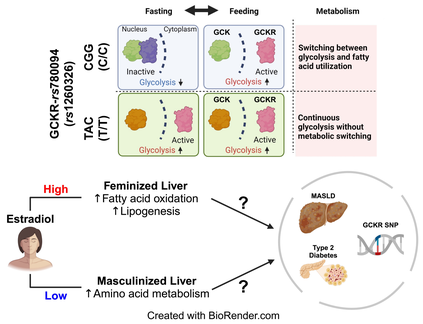
Introduction

The **GCKR rs1260326:C>T** SNP has varying effects on metabolic dysfunction-associated steatotic liver disease (MASLD) pathogenesis in a metabolism-dependent manner[1,2]

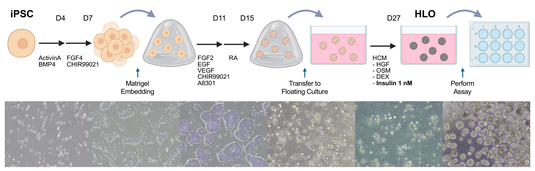
Given that sex-specific differences in metabolism are in part mediated by estrogen receptor-α priming the liver to increase lipogenesis and fatty acid oxidation rather than amino acid metabolism[3,4,5], we hypothesized that hepatic dysfunction due to the GCKR risk allele may be influenced by estradiol stimulation

We investigated the interaction between hepatic metabolism, the GCKR risk allele, and estradiol (E2) stimulation using a human liver organoid (HLO) model derived from isogenic induced pluripotent cell (iPSC) lines and the All of Us genomics dataset



Methods

Whether E2 exposure modifies metabolic response in GCKR risk and non-risk liver organoids[6,7]

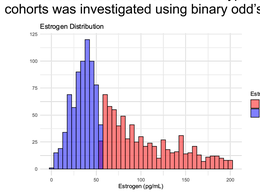


Assay 1: HLO stimulation with 1 or 10 nM E2 for 48 hours[8,9,10,11]
Assay 2: HLO stimulation from day 9 or day 15 with 10 nM E2
Assay 3: HLO stimulation with 300 uM oleic acid, 1000 nM E2, or 300 uM oleic acid and 1000 nM E2 for 48 hours[12]

E2 level association in GCKR risk and non-risk people[13]

Females younger than 45 years old were separated by mean estrogen level (threshold = 55 pg/mL) into high and low estrogen cohorts

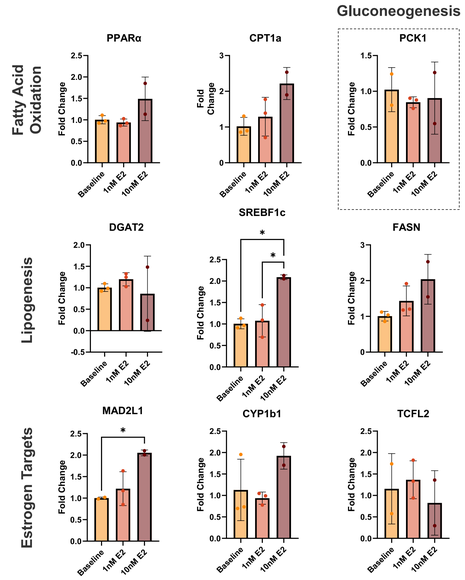
The interaction between steatosis or type 2 diabetes, the GCKR SNP, and estrogen cohorts was investigated using binary odd's ratios and quantitative lab measurements



	Estrogen Category	
	High (>55)	Low(<55)
Participants	975	862
Age, y	37.7 ± 5.4	36.9 ± 5.7
BMI, kg/m2	29.5 ± 8.6	30.6 ± 8.8
Race		
White	575 (59)	459 (53.2)
Black	122 (12.5)	110 (12.8)
Middle Eastern	7 (0.7)	14 (1.6)
Asian	54 (5.5)	47 (5.5)
Other	44 (4.5)	58 (6.7)
Declined to answer	173 (17.7)	174 (20.2)
Condition		
Steatosis	63 (6.5)	78 (9)
Type 2 Diabetes	82 (8.4)	108 (12.5)

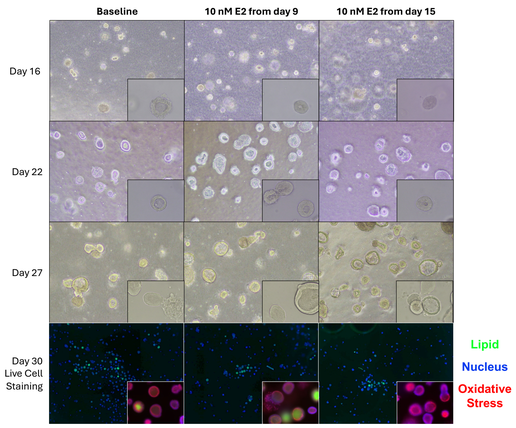
Results

E2 Dose Dependency



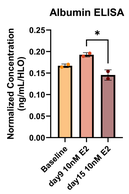
RT-qPCR of cDNA from HLO RNA demonstrated that several fatty acid oxidation and lipogenesis genes increased proportional to E2 stimulation

E2 Long Exposure



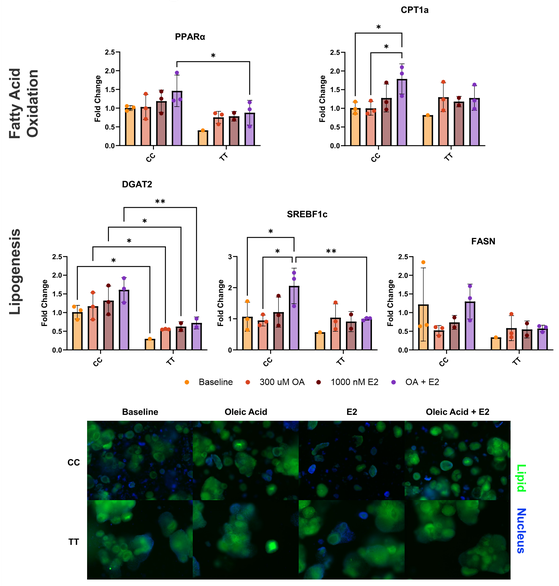
All organoids exposed to E2 demonstrated a more cystic morphology than baseline HLOs

Baseline and day 15 exposed HLOs generated similar albumin levels, whereas day 9 exposed HLOs generated more albumin



Results

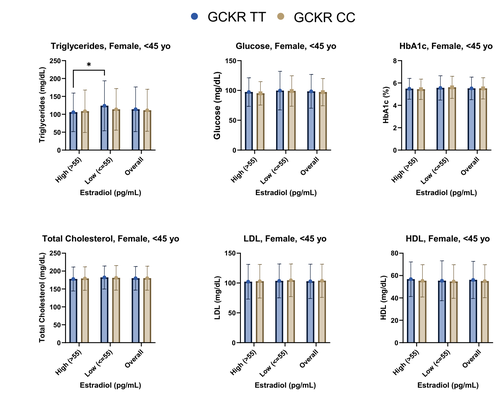
Lipid Loading



E2 exposure primed GCKR CC, but not TT, organoids to increased expression of fatty acid oxidation and lipogenesis genes

GCKR-E2-Metabolism Epidemiology

		T2D						Steatosis					
		+	-	+/-	Low/High	TT/CC		+	-	+/-	Low/High	TT/CC
GCKR TT	Estradiol (pg/mL)												
	High (>55)	28	262	0.107	1.285	**0.709**	High (>55)	21	269	0.078	1.453	**1.262**
	Low (<=55)	32	233	0.137			Low (<=55)	27	238	0.113		
		+	-	+/-	Low/High			+	-	+/-	Low/High	
GCKR CC	Estradiol (pg/mL)											
	High (>55)	38	390	0.097	1.813		High (>55)	33	395	0.084	1.152	
	Low (<=55)	65	368	0.177			Low (<=55)	38	395	0.096		



The GCKR SNP and mean estradiol levels paradoxically affected risk of T2D and steatosis

There was a significant difference in mean triglyceride levels between high and low estradiol GCKR TT patients

Conclusions

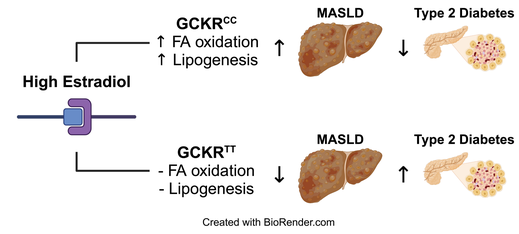
Estradiol proportionally increased fatty acid anabolic and catabolic pathways in the GCKR CC condition

Long term stimulation of estradiol during HLO development promoted a more cystic HLO morphology

The lipid loading study suggests that GCKR TT reduced the plasticity of the liver metabolic response to estradiol stimulation when compared to GCKR CC

These results indicate a complex interplay among estradiol stimulation, genetics, and hepatic metabolism

Further studies are needed to explore the molecular mechanisms by which estradiol-induced metabolic responses can be modified by GCKR and other genotypes



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