Circadian pathways as a mediator between alcohol and liver disease

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Talk Highlights

• Alcohol-induced fatty liver disease

• Energy programs - Glycogen, lipid, and mitochondrial metabolism in the liver

• Dynamic nature of liver metabolism & molecular circadian clock

• Alcohol disrupts the liver circadian clock

• Alcohol disrupts diurnal rhythms in energy metabolism processes – Lipid and Glycogen

• Impact genetic disruption of the clock has on metabolism
  • Hepatocyte-specific BMAL1 knockout mouse model
Alcohol use & Alcoholic liver disease


- Half of Americans drink alcohol

- 15 million heavy drinkers and 52 million binge drinkers

- Most prevalent cause of liver-related illness and death

- 2 million alcohol-related liver disease

- 15,000 - 20,000 deaths/year

- Increasing incidence of liver cancer

- No FDA approved therapies
Alcoholic liver disease – complex spectrum of pathologies

**Early disease**
- **Steatosis:** Fat accumulation, Triglycerides

**Molecular Mechanisms of ALD:**
- Lipid and glycogen alterations
- Oxidative stress | Redox signaling
- Mitochondrial damage | Bioenergetic stress
- Inflammation | Fibrogenesis
- Circadian clocks

**Steatohepatitis:** Fat accumulation, Inflammation, Cell death

**Late disease**
- **Fibrosis/Cirrhosis:** Excess ECM - Collagen

- Molecular Mechanisms of ALD:
  - Lipid and glycogen alterations
  - Oxidative stress | Redox signaling
  - Mitochondrial damage | Bioenergetic stress
  - Inflammation | Fibrogenesis
  - Circadian clocks
Alcohol disrupts energy metabolism pathways

**Lipid - Triglyceride**

**Carbohydrate - Glycogen**

**Mitochondria - Bioenergetics**

**Lipid and CHO Metabolism:**
- Increased fat
- Decreased glycogen

**Bioenergetics:**
- Decreased respiration and ATP
- mtDNA damage | Altered proteome

- Endpoints measured at one time point – ‘Snapshot’ of metabolism
- Liver metabolism is **dynamic** and changes during the day
- Liver is a **different organ** at **different times of the day**
Time-of-day dependent changes in metabolism

Extrinsic and Intrinsic Factors

Many diseases are associated with a ‘broken’ clock

Desynchrony of Rhythms:
Disrupted phase relationships

“Mismatch” in metabolism = Disease
Susceptible population - Shift workers

What about alcohol use disorders and pathologies?

- Heart disease
- Dyslipidemia
- Hypertension
- Obesity
- Diabetes
- Insulin resistance
- Inflammation
- Cancer
Disruption in circadian rhythms and alcoholism


- Clock gene polymorphisms in alcohol use disorders and alcohol consumption. Kovanen et al (2010), *Alcohol Alcohol*

- Increased alcohol consumption in shift work and long working hours. Schulter et al (2012), *Int J Nurs Stud*
  Virtanen et al (2015), *BMJ*

- Reduced expression of circadian clock genes in male alcoholic patients. Huang et al (2010), *Alcohol Clin Exp Res*

**Goal:** Demonstrate the importance of disrupted clocks and metabolic rhythms in alcoholic liver disease

- Does alcohol consumption disrupt the liver molecular clock?

- What effect does chronic alcohol have on diurnal rhythms in energy metabolism?
  - Lipid, glycogen, and mitochondrial metabolism

- Are these processes regulated by the liver clock?
  - Hepatocyte-specific BMAL1 Knockout (HBK) Mouse

- What impact does a disrupted clock have on alcohol-related changes in metabolism?
Male mice: C57BL/6J mice
Hepatocyte specific BMAL1 KO
Control littermates

Feeding Protocol – 5 wk
Collect Tissues: 4 hr for 24 hr

Diurnal oscillations
(12 : 12 hr LD cycle)

Control Diet

Alcohol Diet

3% or 4% ethanol (w/v)
22% or 29% daily calories
Iso-caloric pair-fed controls
Diets = 1 kcal/mL

Lights on

Lights off

Six separate groups of mice
ZT 3, 7, 11, 15, 19, and 23
Chronic alcohol alters clock gene rhythms in liver

**BMAL1 : CLOCK**

<table>
<thead>
<tr>
<th>Gene</th>
<th>BMAL1</th>
<th>CLOCK</th>
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<td>E-box</td>
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**Per1-3**

**Cry1-2**

**Rev-erba**

**Dbp**

**Alcohol disrupts:**
Mesor & amplitude of all clock genes
Acrophase of Cry1, Per2, Rev-erba

*From: Filiano et al. PLoS One 2013*
Alcohol and the Molecular Clock

• Chronic alcohol does not alter the central SCN clock – no effect on clock gene rhythms

• Chronic alcohol influences the liver clock - alters mean expression, amplitude, and phase (timing) of clock genes

• Chronic alcohol advances the liver clock
  • SCN and Liver explants from Per2::Luc mice fed control and alcohol diets
  • Per2 peaks earlier (~1.5 hr) in liver of alcohol-fed mice
  • No effect on SCN Per2 rhythms

• Chronic alcohol consumption desynchronizes the liver clock from the central clock in the SCN

• Circadian desynchrony may contribute to alcoholic liver injury
Chronic alcohol increases lipid and depletes glycogen in liver

Our model: Lieber-DeCarli mouse model of ALD
Steatosis w/ mild inflammation
Earliest stage of ALD

‘Snapshot’ of metabolism – Early morning, ZT 3-4
Inactive/sleep phase
Alcohol disrupts the diurnal rhythm in liver triglyceride.

Diet, p<0.001  
Time, p=0.011  
D X T, p<0.001

n = 4-6 mice/time-point/group
Summary – Fatty acid trafficking genes in liver

Many, many more targets...

Unpublished data

From: DG Mashek, Adv Nutr 2013, 4
Glycogen is the storage form of glucose in mammalian cells. Stored predominantly in muscle and liver, liver glycogen serves two major functions: maintenance of blood glucose level and fuel reserve for ATP synthesis.
Diurnal rhythms in glucose and glycogen metabolism genes

From: Udoh et al, AJP G&L, 2015
Alcohol disrupts the diurnal rhythm in liver glycogen

From: Udoh et al, AJP G&L, 2015

n = 4-6 mice/time-point/group
Alcohol disrupts diurnal rhythm in liver glycogen

Glycogen particle – Dynamic organelle – 40-50 proteins

- Glycogen-targeted phosphatase – Protein Phosphatase 1 – PP1
- GP and GS activities regulated by (de)phosphorylation
  - pGP – active & pGS – inactive
  - GP – inactive & GS – active
- Glycogen targeting proteins – Seven GTPs
  - Scaffold proteins - target PP1 to glycogen particle

From: Roach et al., Biochem J, 2012

Unpublished data
Alcohol depletes liver glycogen

Glycogen metabolism genes exhibit diurnal rhythms

Rhythms are disrupted by chronic alcohol - ↓ Glycogen
What effect does genetic disruption of the clock have on glycogen metabolism?

**Hepatocyte-specific BMAL1 Knockout Mouse Model – HBK mouse**

Chow-fed mice – 12 wks of age, male

**Liver gene expression – Clock-controlled genes**

**BMAL1 Protein in Liver:**

- WT
- HBK
- WT
- HBK
- WT
- HBK

n = 3-4 mice/time-point/group
Alcohol and clock-mediated change in hepatic glycogen

Unpublished data

n = 4-7 mice/time-point/group
Clock dependence of glycogen metabolism genes in liver

3 Factor ANOVA Results – Alcohol Feeding Study
H-BMAL1 KO and control littermates – 5 weeks

Unpublished data
Summary – Alcohol disrupts energy metabolism

Alcohol Consumption

Time of day

DeSynchrony = Disease

Triglyceride Accumulation
Glycogen Depletion
Fatty Liver Disease
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