Final Exam
PMY 406/512/516
• Friday May 19\textsuperscript{th} in Kapoor 190 from 8-11am
• Exam is Comprehensive & Optional
  – Replace lowest of 5 hourly exam
  – OR function as make-up exam for missed exam with approved excuse
  – Allowed single colored crib sheet
    • Available in Pharmacology Office (102 Farber Hall) from May 9-12\textsuperscript{th}
    • Must be hand-written ONLY
    • Can write on both sides
Ethanol
Gift or Curse from the gods?

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Ethanol: Learning Objective and Knowledge Points

• Be able to explain the absorption, metabolism, distribution and excretion (i.e., ADME) of ethanol. Be able to explain first pass metabolism and how it affects blood alcohol levels.

• Be able to describe the steps for each of the three pathways involved in oxidative metabolism of ethanol.

• Be able to list the pathways involved in non-oxidative ethanol metabolism and the role of these pathways in the effects of ethanol.

• Be able to explain how pharmacogenetic differences alter ethanol metabolism.

• Be able to list which drugs are used for emergency treatment of ethanol withdrawal and be able to explain why they are used.

• Be able to lists the mechanisms involved in the acute behavioral effects of ethanol.
Ethanol: Learning Objective and Knowledge Points

• Know that alcohol use disorders (AUD) are a complex heterogeneous set of disorders that are multi-factorial and polygenic.

• Be able to list which drugs are used to treat alcohol use disorders and be able to explain why they are used.

• Be able to discuss the effects of ethanol on the risk for type 2 diabetes, heart disease, and stroke

• Be able to discuss how both acute and chronic ethanol consumption may alter the response to therapeutic agents

• Be able to discuss the different mechanisms involved in the toxic effects of ethanol.

• Be able to discuss alcoholic liver disease (ALD) including the mechanisms involved in the development of ALD

• Be able to discuss ethanol and cancer including possible mechanism for the carcinogenesis
Alcohol Use
2015 National Survey on Drug Use & Health

People ages 18 or older:
• 86.4 % drank at some point in their lifetime
• 56.0 reported drinking in past month
• 26.9% reported binge drinking in last month

Full-time college students ages 18-22:
• 58.0 % drank in past month
• 37.9% reported binge drinking in past month
• 12.5% reported heavy alcohol use in past month

_Binge drinking:_ 5 or more drinks for male & 4 or more drinks for female on same occasion

_Heavy alcohol use:_ binge drinking on 5 or more days in past month
Ethyl alcohol  
Ethanol (EtOH)

- Infinitely soluble in water and slight soluble in lipids
- Freely diffusible across cell membranes
  - Lacks an osmotic effect in biological systems
- Energy source = 7.1 Kcal/gm which is greater than protein or carbohydrates
  - On average ~50% of alcoholic’s calories from EtOH

CH₃CH₂OH
Alcohol levels

• Concentration reported as:
  – vol % (ml EtOH/100ml)
  – wt % (g EtOH/100 ml; also mg%)
  – Molarity (mmoles/L)
  – Proof (twice vol %)

• Blood alcohol concentration (BAL) usually as wt%

Density: 0.7893 g/ml
MW: 46.07 g/mole

“A Drink” operational defined as:
  – 5 oz glass of wine (12%, v/v)
  – 12 oz beer (5%, v/v)
  – 1.5 oz 80-proof (i.e., 40%, v/v)

## Alcohol content of some OTC medications

<table>
<thead>
<tr>
<th>Medication</th>
<th>% alcohol</th>
<th>Medication</th>
<th>% alcohol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formula 44D</td>
<td>20</td>
<td>Cepacol</td>
<td>14</td>
</tr>
<tr>
<td>Nyquil</td>
<td>10</td>
<td>Cogate-100 mouthwash</td>
<td>15</td>
</tr>
<tr>
<td>Nyquil cough</td>
<td>25</td>
<td>Listerine</td>
<td>26</td>
</tr>
<tr>
<td>DayQuil</td>
<td>10-25</td>
<td>Scope</td>
<td>18.5</td>
</tr>
<tr>
<td>Contact Severe Cold</td>
<td>25</td>
<td>Dr Tichener’s mouthwash</td>
<td>70</td>
</tr>
<tr>
<td>Tylenol extra strength liquid &amp; drops</td>
<td>8.5</td>
<td>Geritol</td>
<td>12</td>
</tr>
<tr>
<td>Benedryl</td>
<td>14</td>
<td>Ambesol</td>
<td>70</td>
</tr>
</tbody>
</table>
Pharmacokinetics

- Absorption is by diffusion
  - Less readily from stomach, but rapidly from small intestine
  - Rate of absorption depends on gastric emptying
- Distributed in total body water
  - Rate of distribution & equilibration amongst tissues depends on vascularization & blood flow
  - Very little enters fat
  - Crosses placenta and enters fetus
- Undergoes 1st pass elimination
Elimination

• Alcohol primarily eliminated by oxidative metabolism
  – Minor amount by non-oxidative metabolism

• Hepatic metabolism is primary route of elimination
  – 90-98% ingested dose metabolized by liver
  – Metabolic contribution of other tissues is small, BUT may contribute to toxic effects

• Small percentage excreted unchanged in urine, breath, and sweat
Oxidative alcohol metabolism

Deitrich et al., Alcohol Res Health 29:266-273, 2006
Alcohol dehydrogenase (ADH)

- Major pathway of oxidative alcohol metabolism
- Zero-order kinetics
- ADH is family of cytosolic enzyme
  — Active enzyme composed of 2 subunits
  — 7 genes (divided into 5 classes) with different kinetic properties & displaying significant functional polymorphisms
## ADH isoforms & polymorphisms

<table>
<thead>
<tr>
<th>Gene</th>
<th>polymorphism</th>
<th>protein</th>
<th>EtOH Km (mM)</th>
<th>Vmax (min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADH1A</td>
<td></td>
<td>α</td>
<td>4</td>
<td>30</td>
</tr>
<tr>
<td>ADH1B*1</td>
<td>Arg48, Arg 370</td>
<td>β1</td>
<td>0.05</td>
<td>4</td>
</tr>
<tr>
<td>ADH1B*2</td>
<td>His48, Arg370</td>
<td>β2</td>
<td>0.9</td>
<td>350</td>
</tr>
<tr>
<td>ADH1B*3</td>
<td>Arg48, Cys370</td>
<td>β3</td>
<td>40</td>
<td>300</td>
</tr>
<tr>
<td>ADH1C*1</td>
<td>Arg272, Ile350</td>
<td>γ1</td>
<td>1</td>
<td>90</td>
</tr>
<tr>
<td>ADH1C*2</td>
<td>Gln272, Val350</td>
<td>γ2</td>
<td>0.6</td>
<td>40</td>
</tr>
<tr>
<td>ADH4</td>
<td></td>
<td>π</td>
<td>30</td>
<td>20</td>
</tr>
<tr>
<td>ADH5</td>
<td></td>
<td>χ</td>
<td>&gt;1000</td>
<td>100</td>
</tr>
<tr>
<td>ADH7</td>
<td></td>
<td>σ</td>
<td>30</td>
<td>1800</td>
</tr>
</tbody>
</table>

- All ADH genes expressed in adult liver except *ADH7*
- Point of reference: BAL of 0.08% (w/v) = 17.4mM
- *ADH1A, ADH1B, & ADH1C* account for most of EtOH oxidizing capacity in liver
- Oxidative capacity depends on which alleles are expressed
Aldehyde dehydrogenase (ALDH)

- Acetaldehyde (µM) \[\leftrightarrow\] Acetate (mM) \[\leftrightarrow\] NAD\(^+\) \[\leftrightarrow\] NADH

- Acetaldehyde highly toxic causing aversive reaction from facial flushing, sweating, headache, nausea & tachycardia to severe CV collapse & convulsions
  - Mast cell degranulation & histamine release \[\Rightarrow\] flushing, tachycardia, nausea, bronchoconstriction
  - Forms proteins & DNA adducts

- 18 genes encode ALDH enzyme superfamily

- Most of acetate generated leaves liver
  - Oxidized by other tissue to CO\(_2\) and water
  - Converted to acetyl CoA for lipid and cholesterol biosynthesis
## ALDH isoforms & polymorphisms

<table>
<thead>
<tr>
<th>Gene</th>
<th>polymorphism</th>
<th>Km (μM)</th>
<th>Vmax (min⁻¹)</th>
<th>Vmax (min⁻¹ μM⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALDH1A1</td>
<td></td>
<td>180</td>
<td>380</td>
<td>2.1</td>
</tr>
<tr>
<td>ALDH1B1</td>
<td></td>
<td>55</td>
<td>40</td>
<td>0.7</td>
</tr>
<tr>
<td>ALDH2*1</td>
<td>Lys504</td>
<td>0.2</td>
<td>280</td>
<td>1400</td>
</tr>
<tr>
<td>ALDH2*2</td>
<td>Glu504</td>
<td>1.4</td>
<td>20</td>
<td>14</td>
</tr>
</tbody>
</table>

- [acetaldehyde] usually ~ 5 μM
- ALDH inhibited by Disulfiram (Antabuse)
- Some medications induce disulfiram-like reactions, e.g.,
  - Chlorpropamide
  - Cefotetan
  - metronidazole
Pharmacogenetics of EtOH metabolism
Effect of ADH & ALDH variants on EtOH metabolism

**ADH1B*2**
- enzyme activity $\beta_2$ subunit $> \beta_1$
- Major allele in East Asians
  - Frequency is 75% among Japanese & Chinese
- 20% frequency in Middle East
- Uncommon in European & Africans

**ADH1B*3**
- Enzyme activity $\beta_3$ subunit $> \beta_1$, but high Km
- Relatively common in East Africans
- Allele is rare elsewhere

**ALDH2*2**
- protein is nearly inactive
- Relatively common in Chinese, Japanese & Koreans
- Essentially absent in Europeans & Africans
Cytochrome P450

EtOH $\xrightarrow{\text{CYP2E1}}$ Acetaldehyde

NADPH $\rightarrow$ NADP$^+$

H$^+$ $\rightarrow$ O$_2$ $\rightarrow$ 2 H$_2$O

• Primarily CYP2E1 but also CYP1A2 & CYP3A4
• Important other tissues (e.g., brain) where ADH activity is low
  – CYP2E1 $K_m$ for EtOH of 8-10 mM vs. ADH $K_m$ for EtOH of 0.2-2 mM
• Reaction produces reactive oxygen species (ROS):
  – superoxide anion, hydroxyl radicals, hydroxyethyl radical
• Chronic alcohol consumption induces CYP2E1
  • 4-10X difference in degree of induction
Catalase located in peroxisomes

- Requires hydrogen peroxide (H$_2$O$_2$)-generating system (e.g., xanthine oxidase, NADPH oxidase)

- Minor pathway for alcohol oxidation except in fasted state
Nonoxidative alcohol metabolism

- Minimal contribution to metabolism
  - PLD has high $K_m$ for EtOH
- FAEE have significant pathological effects
- Liver & pancreas have highest levels FAEE synthase & FAEE

Zakhari, Alcohol Res & Health 29:245-254, 2006
Acute EtOH causes CNS depression with anxiolytic actions; behavioral disinhibition
Acute EtOH affects various neurotransmitter, 2\textsuperscript{nd} messengers, & signal transduction pathways

- EtOH increases dopamine, acetylcholine, serotonin, β endorphins release
  - Increase firing rate & bursting activity of ventral tegmental area (VTA) DA neurons projecting to nucleus accumbens (NAcc) & prefrontal cortex
  - Systems involved in reward, motivation, memory, cognition
- Enhances chloride influx by GABA\textsubscript{A} receptor (hyperpolarization)
- Inhibits NMDA-type of glutamate receptors
- Inhibits N- & P/Q-type of voltage-dependent calcium channels
- Enhances adenylyl cyclase activity with ↑cyclic AMP levels and PKA activity
- Activates cannabinoid CB\textsubscript{1} receptors
Repeated administration of EtOH

**Tolerance:** ↓ response with repeated administration; require ↑ doses to elicit same response
- Involves metabolic changes & cellular adaptations

**Physical dependence:** Adaptive state that develops to the repeated presence of EtOH & is manifested by presence of withdrawal syndrome:
- Hyperexcitability in mild cases of withdrawal syndrome
- Seizures, toxic psychosis and delirium tremens in severe cases of withdrawal

Emergency treatment of EtOH withdrawal is symptomatic and supportive
- Longer acting benzodiazepines (e.g., lorazepam, chlordiazepoxide) to stimulate \( \text{GABA}_A \) receptor to counteract withdrawal hyperexcitability
- Clonidine to reduce enhanced sympathetic nervous system discharge
“Alcohol Lexicon”

**Alcohol abuse**: Pattern of drinking that results in adverse consequences

**Addiction**: chronic, relapsing brain disease that is characterized by:
- compulsive drug seeking and use despite negative consequences
- the loss of control over drug intake

Involves changes in brain structure & how the brain works (i.e., “re-wiring of the brain”)

**Alcohol dependence = alcohol addiction**

**Alcoholism (Alcohol Use Disorder)**: broad term for any drinking of alcohol that results in a problem

- Combination of alcohol abuse & alcohol dependence
Alcohol Abuse

Contributing factor for various health problems, violent crimes, injuries and accidents, lost work and reduced productivity, and negatively impacts families & quality of life

Risk factor in cancers of the mouth, esophagus, pharynx, larynx, liver & breast

Responsible for/involved in:
- 32% of cases of liver cirrhosis
- 29% of esophageal cancer
- 25% of liver cancers
- 24% of homicides
- 20% of motor vehicle accidents
- 31% of overall driving fatalities
- 19% of mouth & oropharyngeal cancers
- 11% of suicides
**DMS-5: Alcohol Use Disorder (AUD)**

| 1 | Alcohol is often taken in larger amounts or over a longer period than was intended. (See DSM-IV, criterion 7.) |
| 2 | There is a persistent desire or unsuccessful efforts to cut down or control alcohol use. (See DSM-IV, criterion 8.) |
| 3 | A great deal of time is spent in activities necessary to obtain alcohol, use alcohol, or recover from its effects. (See DSM-IV, criterion 9.) |
| 4 | Craving, or a strong desire or urge to use alcohol. **This is new to DSM-5** |
| 5 | Recurrent alcohol use resulting in a failure to fulfill major role obligations at work, school, or home. (See DSM-IV, criterion 1.) |
| 6 | Continued alcohol use despite having persistent or recurrent social or interpersonal problems caused or exacerbated by the effects of alcohol. (See DSM-IV, criterion 4.) |
| 7 | Important social, occupational, or recreational activities are given up or reduced because of alcohol use. (See DSM-IV, criterion 10.) |
| 8 | Recurrent alcohol use in situations in which it is physically hazardous. (See DSM-IV, criterion 2.) |
| 9 | Alcohol use is continued despite knowledge of having a persistent or recurrent physical or psychological problem that is likely to have been caused or exacerbated by alcohol. (See DSM-IV, criterion 11.) |
| 10 | Tolerance, as defined by either of the following:  
   a) A need for markedly increased amounts of alcohol to achieve intoxication or desired effect  
   b) A markedly diminished effect with continued use of the same amount of alcohol (See DSM-IV, criterion 5.) |
| 11 | Withdrawal, as manifested by either of the following:  
   a) The characteristic withdrawal syndrome for alcohol (refer to criteria A and B of the criteria set for alcohol withdrawal)  
   b) Alcohol (or a closely related substance, such as a benzodiazepine) is taken to relieve or avoid withdrawal symptoms. (See DSM-IV, criterion 6.) |

The presence of at least 2 of these symptoms indicates an Alcohol Use Disorder (AUD).

The severity of the AUD is defined as:

- **Mild:** The presence of 2 to 3 symptoms
- **Moderate:** The presence of 4 to 5 symptoms
- **Severe:** The presence of 6 or more symptoms

~20% of college students met criteria for AUD
• Alcohol Use Disorder (AUD) involves multiple factors.

- Biology/Genes
- Environment
- DRUG
- Brain Mechanisms
- AUD

- Alcohol Use Disorder is a complex heterogeneous disorder that is multi-factorial and polygenic.
- Genetic factors account for 40-60% of a person’s vulnerability to alcoholism including the effects of environment on gene expression & function.
Alcohol Use Disorders

2015 National Survey on Drug Use & Health

• In USA 15.1 million adults (18 yr & older) had alcohol use disorder (9.8 million men & 5.3 million women)
  – 623,000 adolescents (ages 12-17) had an alcohol use disorder
• Alcohol is 4\textsuperscript{rd} leading preventable cause of deaths in USA
  – 88,000 die from alcohol-related causes annually
  – 31\% of overall driving fatalities involve alcohol (9967 deaths in 2014)
• Economic burden of alcohol misuse in USA of $249 billion

Global Burden

• 5.9\% of all global deaths (3.3 million deaths) attributed to alcohol consumption
• 5\textsuperscript{th} leading risk factor for premature death and disability
FDA approved treatments for Alcohol Use Disorders

• **Acamprosate** (Campral)
  - Helps prevent relapse in people who have stopped drinking
  - Modulates changes in glutamatergic and GABAergic activity associated with chronic EtOH use
  - Does not prevent withdrawal

• **Naltrexone**
  - Opioid receptor antagonist
  - Endogenous opioids involved in reinforcement pathways

• **Disulfiram** (Antabus)
  - Inhibitor of acetaldehyde dehydrogenase
  - ↑ acetaldehyde levels during drinking causes aversive symptoms (e.g., nausea, profuse vomiting, flushing, sweating, palpitations, dyspnea)
  - Poor compliance unless highly motivated
  - Potential problem with OTC drugs containing alcohol
Beneficial health effects of alcohol consumption?

Moderate alcohol consumption (i.e., up to 1 drink/day for women and 2/day for men) associated with *Decreased risk* of heart disease, ischemic stroke & type 2 diabetes

- Estimated 26,000 deaths averted in 2005 due to reduction in heart disease, stroke & diabetes

- However, studies suggested confounded by # issues including reference group bias, drinking pattern & reporting of consumption question

- **NOTE:** beneficial effects are dose-dependent with beneficial effects associated with moderate consumption and adverse effects at higher doses
  - J-Shaped relationship for alcohol intake & cardiovascular effects
  - U-shaped relationship for alcohol intake & type 2 diabetes
Putative mechanisms for reduced risk of type 2 diabetes with moderate alcohol intake

- ↑ insulin sensitivity & ↓ fasting insulin levels
- ↑ adiponectin release from adipocytes ⇒ modulates glucose levels and fatty acid oxidation + has anti-inflammatory properties
- ↓ fatty acid release from adipocytes & ↓ fatty acid oxidation
- Favorable effects on glucose metabolism
  - ↑ NADH:NAD⁺ with EtOH metabolism inhibits gluconeogenesis which tend to ↓ blood glucose especially in fasted state
  - With meals, low dose EtOH blunts postprandial glucose spike & subsequent inflammation

BUT U-shape relationship between alcohol intake & risk of type 2 diabetes
Cardiovascular Effects of Alcoholic Beverages

• Protective effects may involve:
  – ↑ HDL
  – ↑ insulin sensitivity & ↓ risk type 2 diabetes
  – ↓ C-reactive protein (i.e., marker of vascular inflammation)
  – ↓ oxidized LDL which is very atherogenic
  – Flavonoids in alcoholic beverages (e.g. red wine) have antioxidant effect
  – ↓ thrombosis (i.e., clotting)
    • ↑ plasminogen activator which dissolves clots
    • ↓ platelet aggregation
    • ↓ reduces fibrinogen levels

• **BUT** high alcohol consumption associated with:
  – Cardiomyopathies due in part to ↑ FAME
  – Arrhythmias (prolongs QT interval & shortens atrial effective refractory period)
  – Hypertension
Alcohol interactions with Therapeutic Compounds

17 of the top 38 most prescribed drugs have specific precautions about alcohol use. WHY the precaution?

➢ EtOH-mediated alteration in Drug Metabolism

- CYP450-mediated EtOH metabolism mainly by CYP2E1 but also by CYP1A2 & CYP3A4
  - Presence of EtOH may compete for drug metabolized by CYP450s
  - Currently, few drugs metabolized by CYP2E1 (e.g., chlorzoxazone), but various drugs metabolized by CYP1A2 (e.g., warfarin, propranolol) and CYP3A4 (e.g., calcium channel blockers, warfarin, & some statins)
- Chronic EtOH consumption increases hepatic CYP2E1 levels → ↑ generation of toxic metabolites
  - acetaminophen ⇒ N-acetyl-p-benzoquinonimine (hepatotoxicity)
  - fluorinated volatile anesthetics (e.g., enflurane, halothane, methoxyflurane, sevoflurane) ⇒ nephrotoxicity
Alcohol interactions with Therapeutic Compounds

- **Effects of EtOH on drug absorption**
  - EtOH can act as co-solvent & increase apparent drug solubility and/or dissolution rate in GI fluids
  - Concurrent intake of EtOH increases solubility of non-ionizable, lipophilic compounds *in vitro*

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*in silico* simulation indicates EtOH ↑plasma drug levels for non-ionizable compounds
EtOH on Drug Absorption

- EtOH-induced ↑ in drug solubility in the GI tract can lead to dose dumping of oral controlled release formulations
- Palladone XL® (1X daily controlled release hydromorphone) withdrawn in 2005 due to EtOH-induced dose dumping
- FDA guidelines suggest testing dissolution behavior of release formulations for 2 hr with 0%, 5%, 20%, & 40%(v/v) EtOH in acidic medium reflecting the gastric milieu
Alcohol interaction with Therapeutic Compounds

Pharmacodynamic interactions

- Potentiation of hypotensive drug action due to BP lowering action of low dose EtOH
  - e.g., Caution with metoprolol, lisinopril
- High dose EtOH prolongs QT interval so increased risk of arrhythmias with drugs that also prolong QT interval
  - e.g., caution with azithromycin, sertraline
- Increased GI bleeding with NSAIDs (e.g., meloxicam, ibuprofen) due to loss of cytoprotective effects of prostaglandins vs. ulcerogenic actions of EtOH/acid:
  - EtOH at low dose stimulates gastric acid secretion, damages parietal cells, damages protective gastric bicarbonate layer, increases gastric mucosal permeability
Pharmacodynamic interactions

- EtOH ↓ thrombosis (i.e., clotting), so possibly increased bleed with anti-coagulants (e.g., warfarin)

- Hypoglycemia with anti-diabetic agents (e.g., metformin)

- Increased sedation, drowsiness, and impaired motor skills due to CNS depressant action of EtOH
  - e.g., Opiates, anti-histamines, tricyclic antidepressants, trazodone

- Synergistic CNS depression with barbiturates & benzodiazepines
Drug Interactions associated with toxic effects of EtOH

• Increased generation of toxic metabolites due to ↑ CYP2E1 with chronic EtOH administration
  – Hepatotoxicity with acetaminophen
  – Nephrotoxicity with fluorinated volatile anesthetics

• Chronic EtOH intake associated with organ damage/dysfunction especially liver and pancreas
  – Alcoholic liver disease (ALD) among top 20 causes of death worldwide.
    • 1 in 3 liver transplants due to ALD
  – Resulting hepatic damage/dysfunction in may impair drug metabolism and drug action (e.g., statins such as pravastatin & simvastatin)
Alcoholic Liver Disease (ALD)

Fatty liver (steatosis): Reversible enlarged liver with lipid accumulation

Steatohepatitis: Swollen liver with inflammation & hepatocyte death by necrosis & apoptosis

Cirrhosis: Stellate cell activation with excess production of extracellular matrix & fibrosis
Toxic effects of EtOH

• *Chronic EtOH exposure alone may not be causative, but rather may sensitize organ to injury*
  – Although <10% of heavy alcohol users develop pancreatitis, alcohol abuse associated with ~35% of cases of acute pancreatitis & ~70% of cases of chronic pancreatitis
  – Only a fraction of chronic alcohol abusers develop cirrhosis

• “Multiple hit”
  – ↑ risk liver damage with isoniazid or duloxetine in alcohol abusers
  – ↑ risk pancreatitis with hydrochlorothiazide in alcohol abusers
Mechanisms involved in toxic effects of EtOH

- **↑ Oxidative Stress**
  - ROS damage proteins, lipids, & DNA → cellular & mitochondrial dysfunction; apoptosis

- **Generation of Acetaldehyde**
  - Protein & DNA adducts → mitochondrial dysfunction

- **Generation of FAEE**
  - Promote cholesteryl esters → destabilized membranes
  - Activate transcription factors →↑ pro-inflammatory cytokines
  - Disrupt intracellular calcium regulation

- **Mitochondrial dysfunction**
  - Damage due to acetaldehyde protein & DNA adducts
  - Damage due to Oxidative stress
  - Altered intracellular calcium regulation → sustained ↑ cytoplasmic Ca\(^{++}\) & mitochondrial Ca\(^{++}\) overload → mitochondrial dysfunction
Mechanisms involved in toxic effects of EtOH

- Altered Signal transduction & gene expression
  - In liver: altered signal transduction & gene expression favor fat synthesis & accumulation over oxidation & transport
  - In pancreas: ↑ cytokine expression → excessive extracellular matrix protein production & fibrosis
  - Altered intracellular calcium regulation → mitochondrial Ca\(^{++}\) overload → mitochondrial dysfunction
  - Alcohol modulates gene expression via changes in noncoding micro RNA (miRNA) and epigenetic modifications of histones & DNA methylation
EtOH modulates immune response in a dose- and time-dependent manner

<table>
<thead>
<tr>
<th>CELL</th>
<th>Moderate Chronic</th>
<th>Acute</th>
<th>Heavy</th>
<th>Chronic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monocyte</td>
<td>↑ Phagocytic activity, ↑ IL-6, TNF-α</td>
<td>↓ Frequency, ↓ IL-6, IL-12, TNF-α, ↓ Effercytosis, ↑ IL-10</td>
<td>↑↑↑ TNF-α</td>
<td></td>
</tr>
<tr>
<td>Dendritic cell</td>
<td></td>
<td>↓ IL-12, ↑ IL-10, ↓ CD80/CD86</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T lymphocyte</td>
<td>↑ Frequency, ↑ IL-2, IL-4, IL-10, IFN-γ, ↓ IFN-γ/IL-10 ratio, ↑ Vaccine responses</td>
<td>↑ Apoptosis</td>
<td>↓ Frequency, ↑ Naive T cells, ↑ Memory T cells, ↑ Activation, ↓ Antigen-specific responses</td>
<td></td>
</tr>
<tr>
<td>B lymphocyte</td>
<td>↑ Frequency, ↓ IgA, IgM, IgG</td>
<td>↑ Apoptosis, ↑ IgA</td>
<td>↓ Frequency, ↑↑↑ IgA, IgM</td>
<td></td>
</tr>
</tbody>
</table>
Chronic EtOH decreases host immune defenses

**Lungs**
- \( \downarrow \) Tight Junctions
- \( \uparrow \) Barrier Permeability
- \( \downarrow \) TLR Responses
- \( \downarrow \) Neutrophil Granule Contents Release
- \( \downarrow \) Phagocytosis
- \( \downarrow \) Chemotaxis
- \( \downarrow \) Pro-inflammatory Cytokines
- \( \uparrow \) Anti-inflammatory Cytokines
- \( \downarrow \) GM-CSF
- \( \downarrow \) Granulopoiesis

**Liver**
- \( \downarrow \) Kupffer Cell Phagocytosis
- \( \uparrow \) Pro-inflammatory Cytokines
- \( \uparrow \) M1 Macrophage Polarization
- \( \downarrow \) M2 Macrophage Polarization
- \( \uparrow \) Reactive Oxygen Species (ROS)
- \( \uparrow \) Leukotrienes

**Spleen**
- \( \downarrow \) T lymphocyte Production
- \( \downarrow \) B lymphocyte Production

**Stomach**
- \( \downarrow \) Gastric Acid Secretion
- \( \uparrow \) Live Bacteria into Intestine

**Small Intestine**
- \( \downarrow \) Tight Junctions
- \( \downarrow \) Zonula occludens-1 (ZO-1)
- \( \uparrow \) Barrier Permeability
- \( \uparrow \) Bacterial Colonization
- \( \downarrow \) Gastrointestinal Motility
- \( \uparrow \) Acetaldehyde Production
- \( \uparrow \) LPS into Bloodstream
Alcoholic fatty liver (steatosis)
Reversible enlarged liver with lipid accumulation

EtOH impairs carbohydrate metabolism and favors fat synthesis & accumulation over oxidation & transport:
• EtOH metabolism $\uparrow$ NADH:NAD$^+$ ratio $\Rightarrow$ Lower NAD$^+$ levels impairs normal carbohydrate metabolism
• EtOH-induced changes in transcription factors $\Rightarrow \uparrow$ enzymes for synthesis of cholesterol & fatty acids; $\downarrow$ transcription of genes involved in oxidation, transport & export of free fatty acids
• EtOH decreases metabolic regulators $\Rightarrow \downarrow$ transport fatty acids into mitochondria, $\downarrow$ oxidation, & $\downarrow$ export of free fatty acids
• EtOH $\uparrow$ ApoB degradation $\rightarrow \downarrow$ secretion lipoproteins
Alcoholic Steatohepatitis

Steatosis with inflammation & hepatocyte death by necrosis & apoptosis

- EtOH stimulates overgrowth of bacteria in GI tract & shifts balance toward more pathogenic species
- EtOH & Acetaldehyde disrupt GI barrier function ⇒ ↑[LPS] portal circulation
- EtOH sensitizes Kupffer cells ⇒ ↑ LPS-induced immune response
- ↑ pro-inflammatory cytokines (e.g., TNFα) & cell death via:
  - Enhanced LPS stimulation of TLR on Kupffer cells
  - ↑ ROS generation
  - Cellular debris from damaged hepatocytes further activates TLR
  - EtOH ↑ activation of complement systems
Alcoholic Cirrhosis
Excess production of extracellular matrix & fibrosis $\Rightarrow$ liver hardening & reduction in hepatic sinusoids; ↑ risk for hepatocellular carcinoma

- EtOH, acetaldehyde, LPS and pro-inflammatory cytokines ↑
  Hepatic stellate cells (HSC) differentiation into myofibroblasts with ↑ production of extracellular matrix (i.e., fibrogenesis)
- ↑ HSC response to pro-fibrogenic factors (TGF-β & PDGF)
- Levels of microRNA29, which normally inhibit extracellular matrix[ECM] production, are reduced
- ROS inhibit metalloproteases that would normally degrade ECM
- Chemokines recruit immune cells (e.g., macrophages) that enhance HSC differentiation into myofibroblasts
- EtOH inhibits anti-fibrotic effect of natural killer (NK) cells & interferon (IFN-γ)
Ethanol & Cancer

Heavy alcohol consumption reported to \( \uparrow \) risk for cancer of oral cavity, throat (pharynx), larynx (voice box), esophagus and liver as well as colorectal and breast cancer.

<table>
<thead>
<tr>
<th>Cancer</th>
<th>Relative Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral &amp; throat</td>
<td>5.13</td>
</tr>
<tr>
<td>esophageal</td>
<td>4.95</td>
</tr>
<tr>
<td>laryngeal</td>
<td>2.65</td>
</tr>
<tr>
<td>colorectal</td>
<td>1.44</td>
</tr>
<tr>
<td>breast</td>
<td>1.61</td>
</tr>
</tbody>
</table>

Effects of light to moderate alcohol consumption, however, are controversial.

Moderate Alcohol consumption suggested associated with decreased risk some blood cancers (e.g., non-Hodgkin’s lymphoma, multiple myeloma), thyroid cancer, renal cell carcinoma.)
Ethanol and Carcinogenesis

- Acetaldehyde classified as carcinogen (Group 1) by International Agency for Research on Cancer
- Direct adduction of acetaldehyde interferes with DNA synthesis & repair enzymes, inhibits DNA methyltransferase and alters histone modifications
- Formation of DNA adducts can be mutagenic (e.g., Cr-PdG)
- Low ALDH2 activity (e.g., heterozygous for ALDH2*2 allele) associated with 11x ↑ relative risk for oropharyngeal/laryngeal cancer and 12.5x ↑ relative risk for esophageal cancer
- GI mucosa contains ADH, but little ALDH2 so little breakdown acetaldehyde
- Gut bacteria form acetaldehyde & their growth stimulated by EtOH
- Acetaldehyde also in cigarette smoke with greater risk if smoke & drink.
Some possible mechanisms for alcohol carcinogenesis

• Acetaldehyde
• Oxidative stress
  – ROS promote DNA damage (e.g., 8-oxo-7,8-dihydroguanine induces DNA base mutation in tumor suppressor gene in liver cancer cells)
  – ROS alters cell signaling & gene expression (e.g., ↓ protection via hypermethylation of promoter for tumor suppressor E-cadherin)
  – Lipid peroxidation products (e.g., 4-hydroxynonenal [4-HNE]) can form DNA adducts that are highly mutagenic & carcinogenic
  – Binding of 4-HNE to p53 (tumor suppressor gene) causes cell to become resistant to apoptosis
• Dysregulation of epigenetic modifications: global hypomethylation, histone modifications, promoter methylation, aberrant expression of non-coding RNA (miRNA)