Final Exam
PMY 406/512/516

- Friday May 19th 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 9-12th
    - 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)

CH_3CH_2OH

- 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

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 :

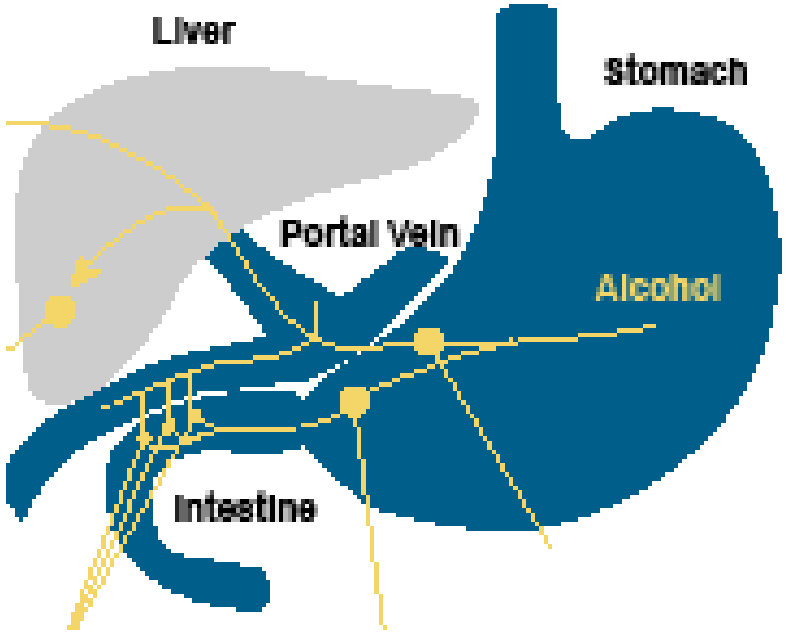
- 5 oz glass of wine (12%, v/v)
- 12 oz beer (5%,v/v)
- 1.5 oz 80-proof (i.e., 40%, v/v)

See "Standardization of Alcohol Calculations in Research" J. Brick. Alcoholism Clin Exp Res 30: 1276-1287, 2006

Alcohol content of some OTC medications

Medication	% alcohol	Medication	% alcohol
Formula 44D	20	Cepacol	14
Nyquil	10	Cogate-100 mouthwash	15
Nyquil cough	25	Listerine	26
DayQuil	10-25	Scope	18.5
Contact Severe Cold	25	Dr Tichener's mouthwash	70
Tylenol extra strength liquid & drops	8.5	Geritol	12
Benedryl	14	Ambesol	70

Pharmacokinetics

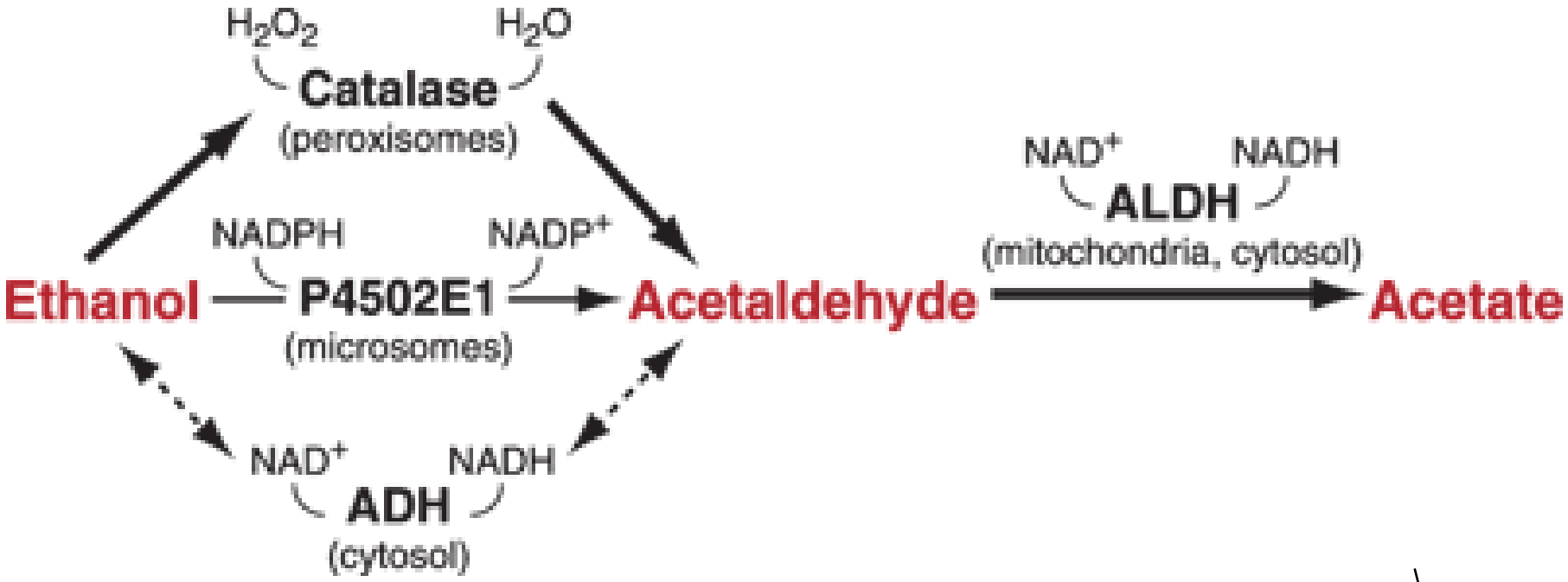


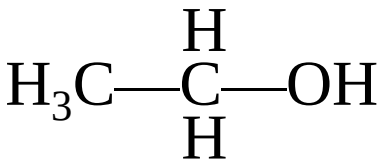
- Absorption is by diffusion
  - Less readily from stomach, but rapidly from small intestine
  - Rate of absorption depends on gastric emptying
- Distributed in total body water
  - Rate of distribution & equilibration amongst tissues depends on vascularization & blood flow
  - Very little enters fat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

Elimination

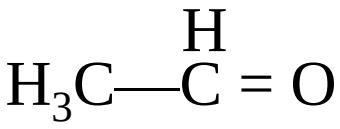
- Alcohol primarily eliminated by oxidative metabolism
  - Minor amount by non-oxidative metabolism
- Hepatic metabolism is primary route of elimination
  - 90-98% ingested dose metabolized by liver
  - Metabolic contribution of other tissues is small, BUT may contribute to toxic effects
- Small percentage excreted unchanged in urine, breath, and sweat

Oxidative alcohol metabolism

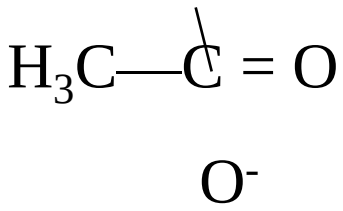




EtOH



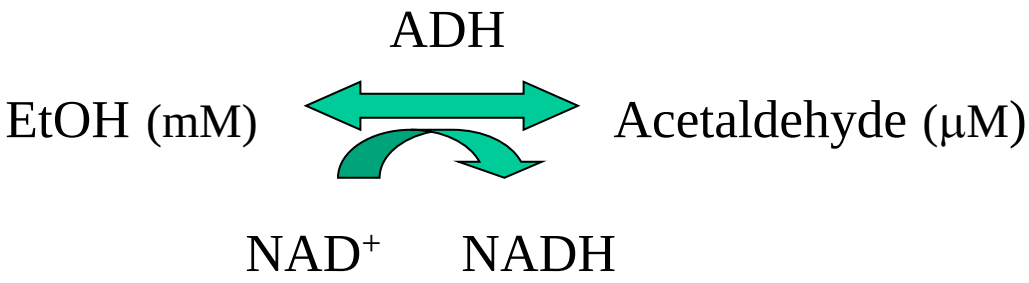
Acetaldehyde



Acetate

Deitrich et al., Alcohol Res Health 29:266-273, 2006

Alcohol dehydrogenase (ADH)



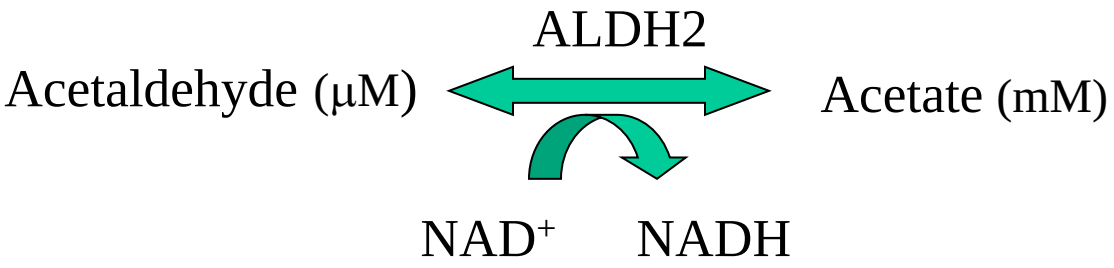
- Major pathway of oxidative alcohol metabolism
- Zero-order kinetics
- ADH is family of cytosolic enzyme
  - — Active enzyme composed of 2 subunits
  - — 7 genes (divided into 5 classes) with different kinetic properties & displaying significant functional polymorphisms

ADH isoforms & polymorphisms

Gene	polymorphism	protein	EtOH Km (mM)	Vmax (min^{-1})
ADH1A		α	4	30
*ADH1B*1*	Arg48, Arg 370	β1	0.05	4
*ADH1B*2*	His48, Arg370	β2	0.9	350
*ADH1B*3*	Arg48, Cys370	β3	40	300
*ADH1C*1*	Arg272, Ile350	γ1	1	90
*ADH1C*2*	Gln272,Val350	γ2	0.6	40
ADH4		π	30	20
ADH5		χ	>1000	100
ADH7		σ	30	1800

- 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 highly toxic causing aversive reaction from facial flushing, sweating, headache, nausea & tachycardia to severe CV collapse & convulsions
  - — Mast cell degranulation & histamine release ⇨ 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

Gene	polymorphism	Km (μM)	Vmax (min^{-1})	Vmax (min^{-1} μM^{-1})
ALDH1A1		180	380	2.1
ALDH1B1		55	40	0.7
*ALDH2*1*	Lys504	0.2	280	1400
*ALDH2*2*	Glu504	1.4	20	14

- [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 β2 subunit > β1
- Major allele in East Asians
  - Frequency is 75% among Japanese & Chinese
- 20% frequency in Middle East
- Uncommon in European & Africans

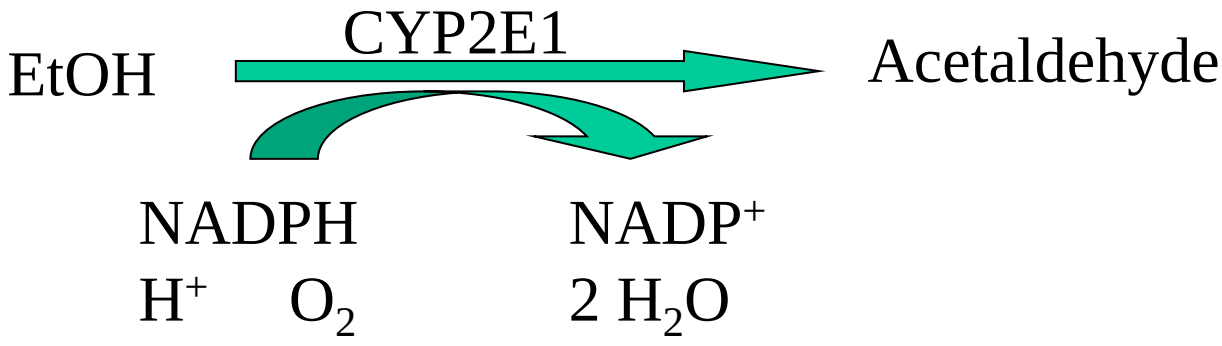
ADH1B*3

- Enzyme activity β3 subunit > β1, but high Km
- Relatively common in East Africans
- Allele is rare elsewhere

ALDH2*2

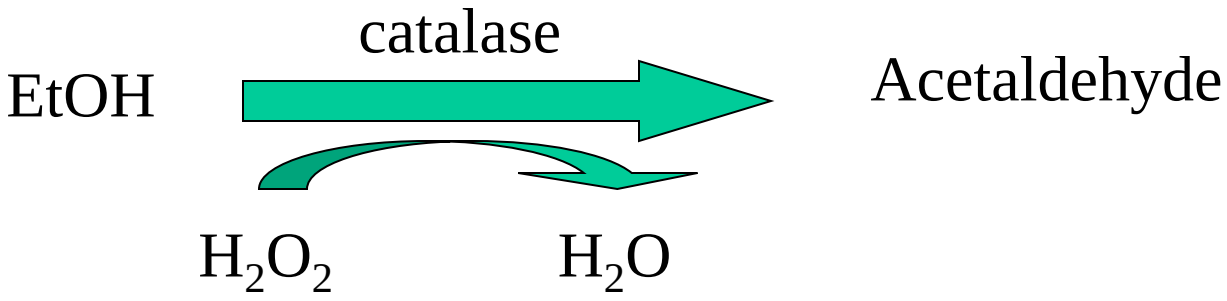
- protein is nearly inactive
- Relatively common in Chinese, Japanese & Koreans
- Essentially absent in Europeans & Africans

Cytochrome P450



- 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

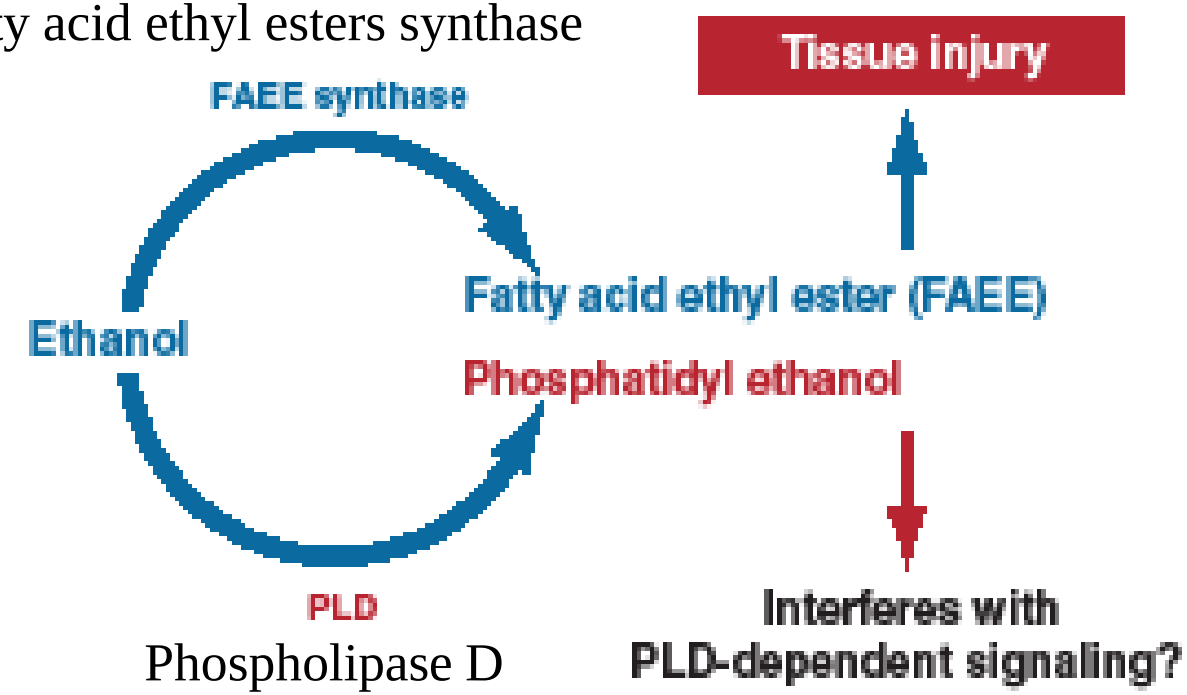
Catalase



- Catalase located in peroxisomes
- Requires hydrogen peroxide (H_2O_2)-generating system (e.g., xanthine oxidase, NADPH oxidase)
- Minor pathway for alcohol oxidation except in fasted state

Nonoxidative alcohol metabolism

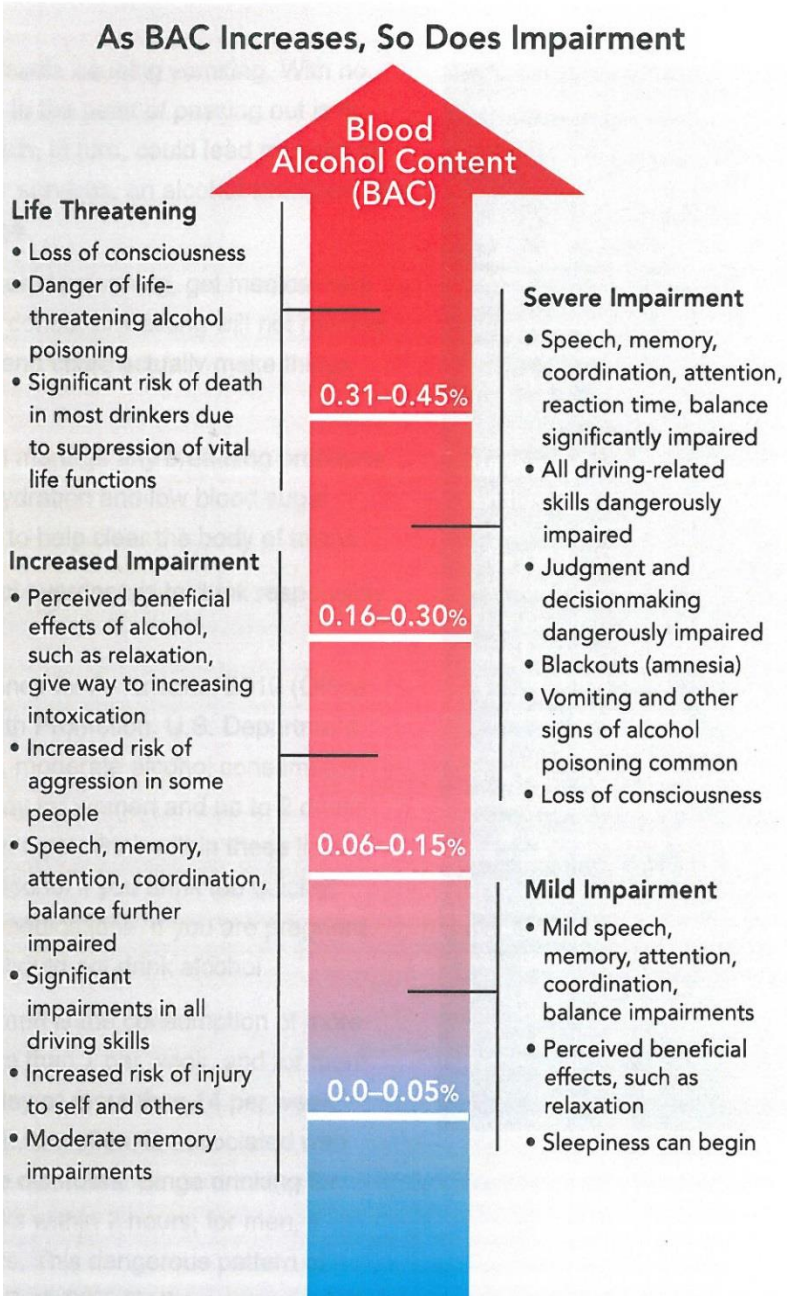
Fatty acid ethyl esters synthase



Phospholipase D

- 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 dis-inhibition

Acute EtOH affects various neurotransmitter, 2nd 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 $GABA_A$ 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_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 $GABA_A$ 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 & oropharygeal 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:

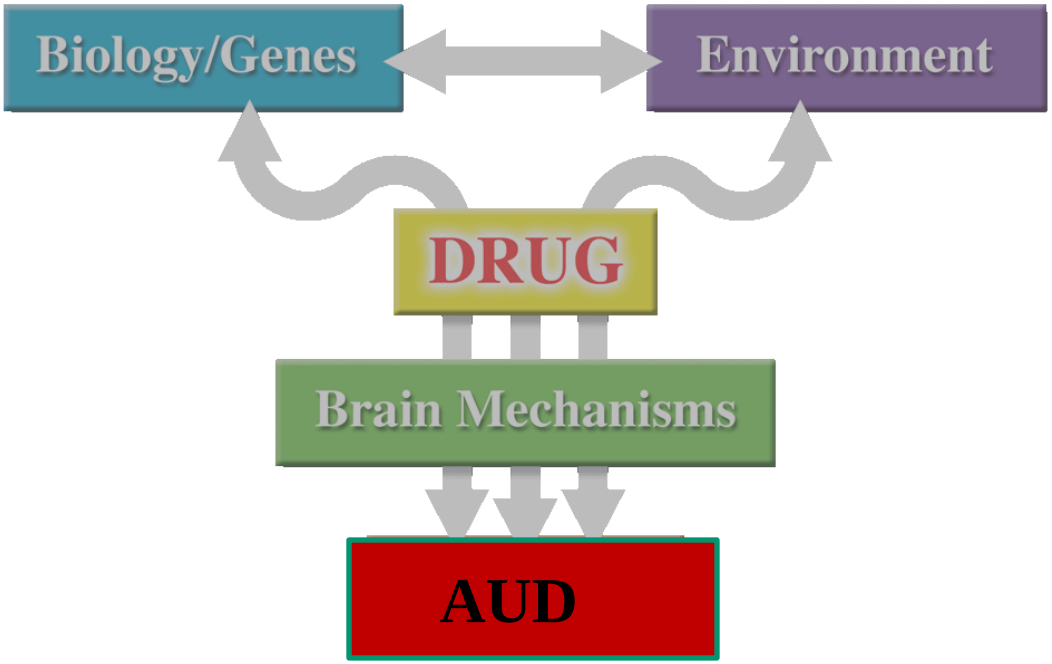
Mild:
The presence of 2 to 3 symptoms

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



- 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 4rd 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
- 5th 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 averse 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 adiocytes & ⇓ 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?

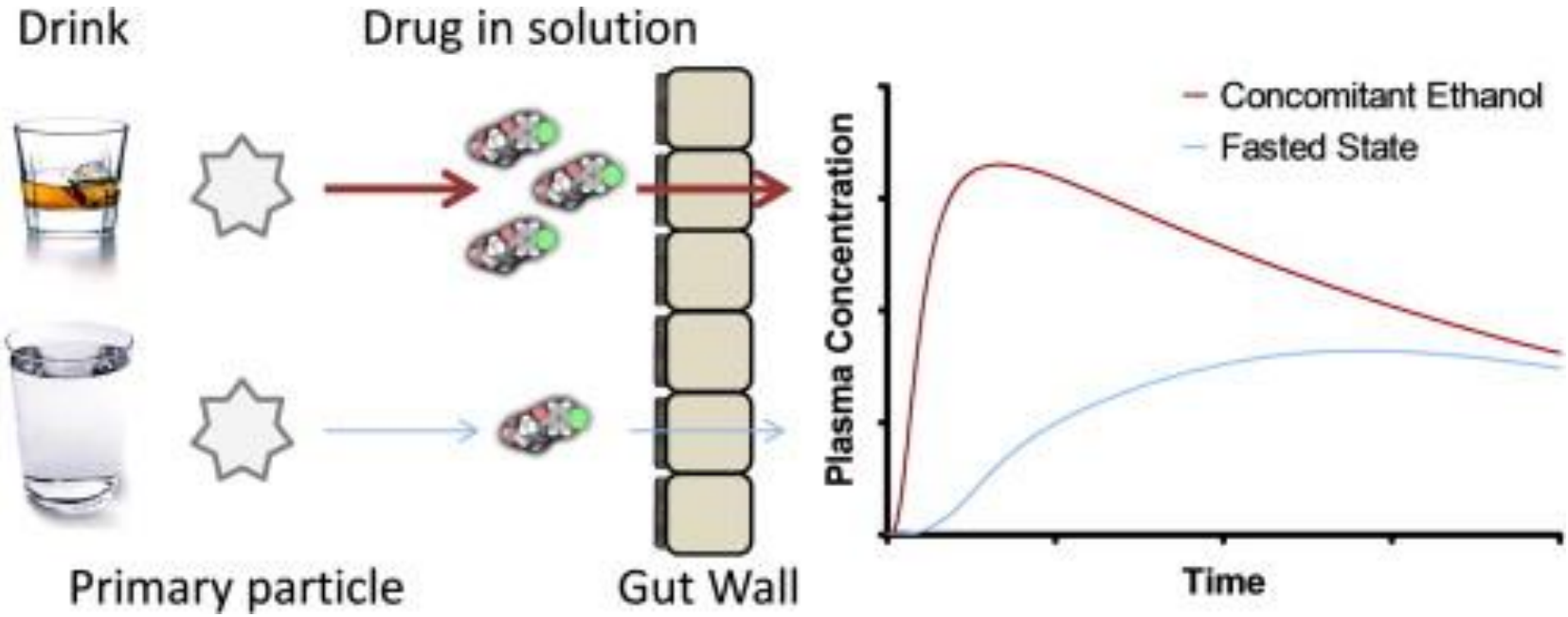
➢ EtOH-mediated alteration in Drug Metabolism

- 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-benzochinonimine (hepatotoxicity)
  - fluorinated volatile anesthetics (e.g., enflurane, halothane, methoxyflurane, sevoflurane) ⇨ nephrotoxicity

Alcohol interactions with Therapeutic Compounds

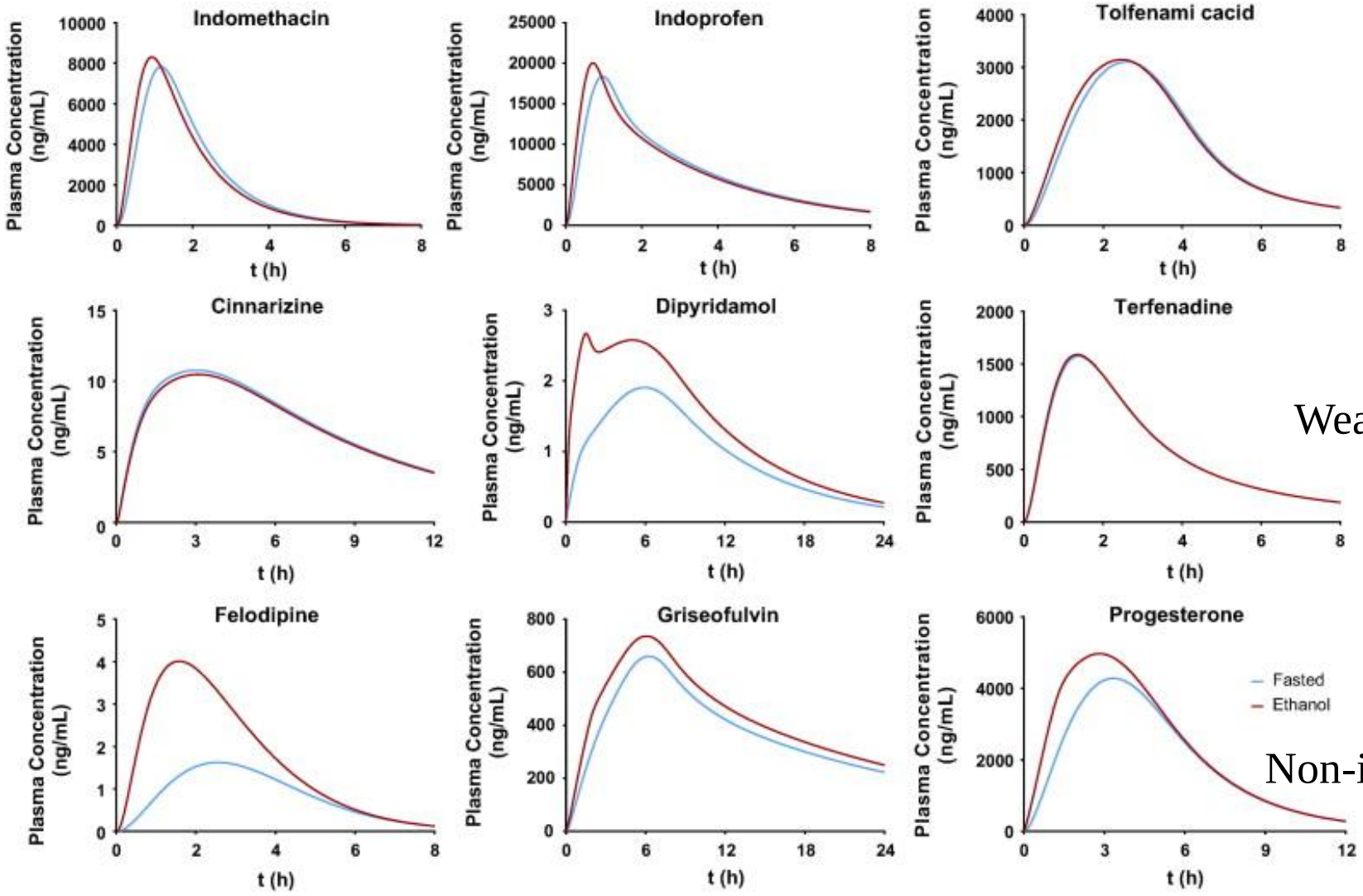
➢ <u>Effects of EtOH on drug absorption</u>

- 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*



Eur J Pharm Sci, 67:12-20, 2015

in silico simulation indicates EtOH ↑plasma drug levels for non-ionizable compounds



acidic

Weak bases

Non-ionizable

Eur J Pharm Sci, Volume 67: 12-20, 2015

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 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

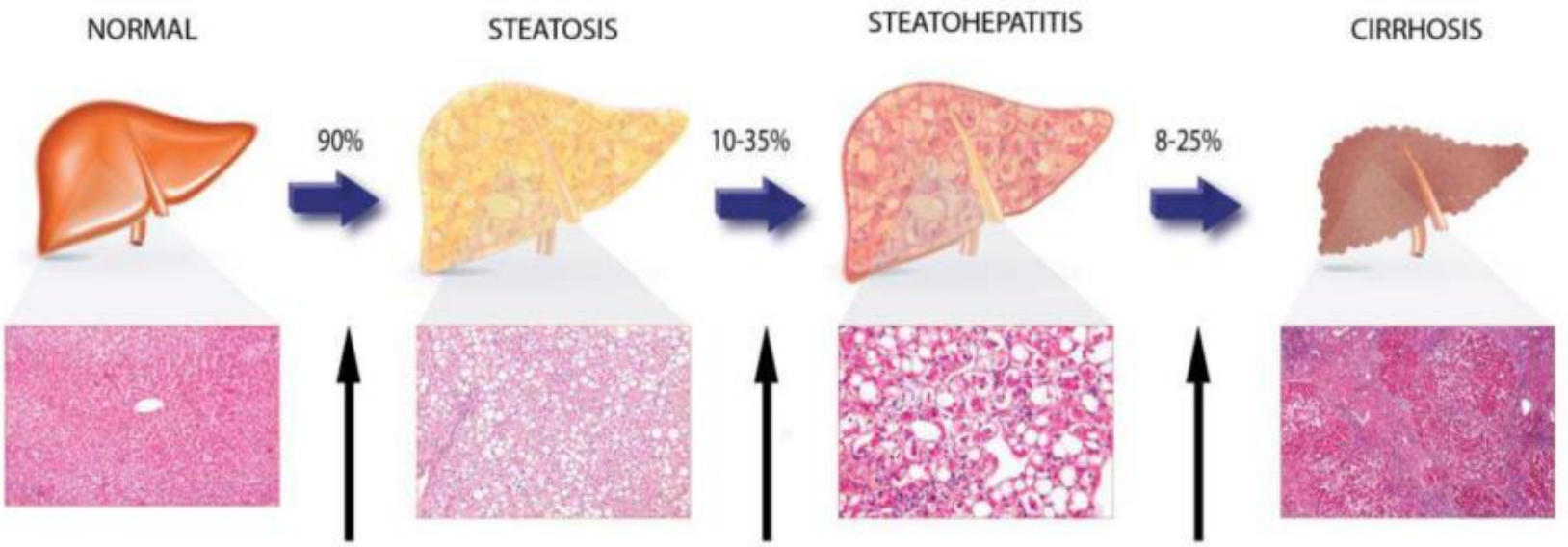
- Synergistic CNS depression with barbiturates & benzodiazepines

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

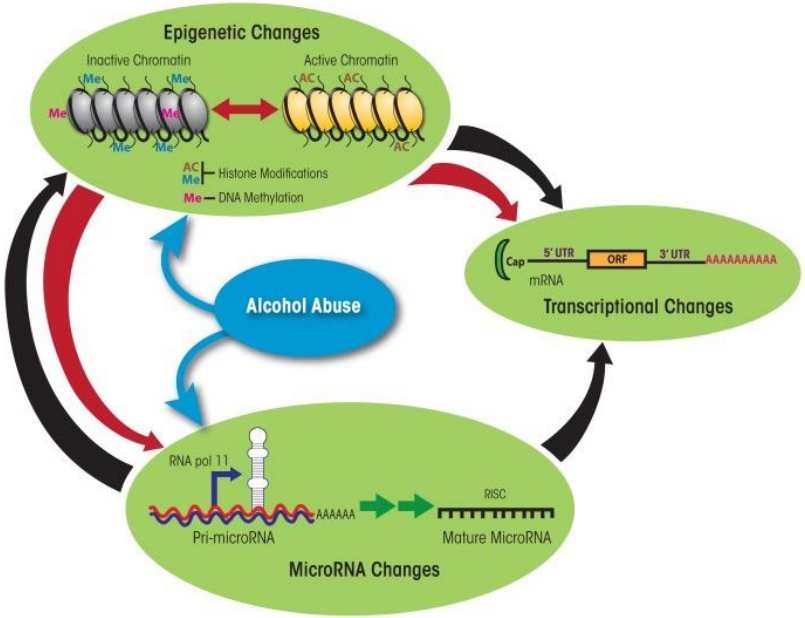
- ⇑ Oxidative Stress
  - ROS damage proteins, lipids, & DNA → cellular & mitochondrial dysfunction; apoptosis
- Generation of Acetaldehyde
  - Protein & DNA adducts → mitochondrial dysfunction
- Generation of FAEE
  - Promote cholesteryl esters → destabilized membranes
  - Activate transcription factors →↑ 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

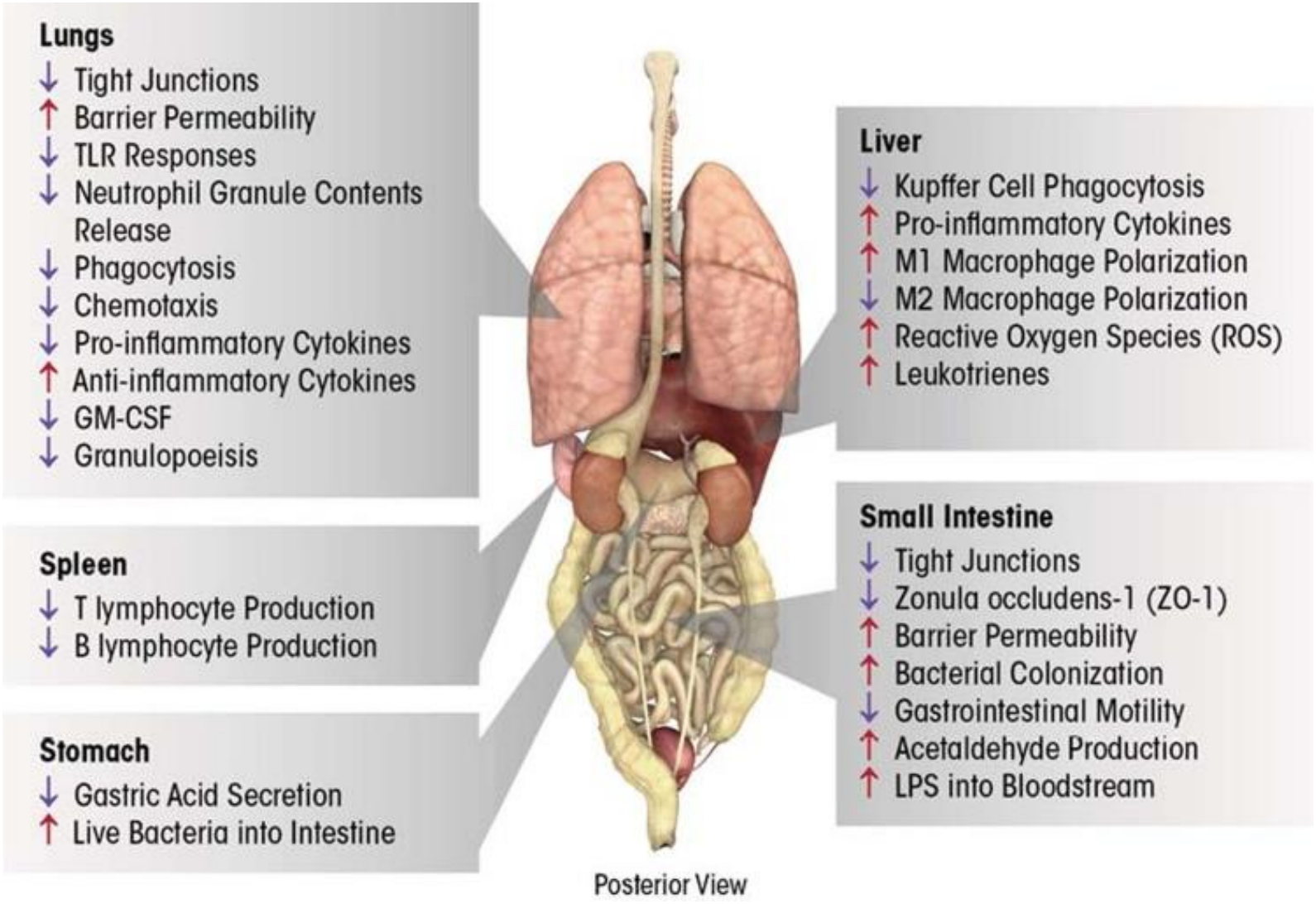
• Alcohol modulates gene expression via changes in noncoding micro RNA (miRNA) and epigenetic modifications of histones & DNA methylation



EtOH modulates immune response in a dose- and time-dependent manner

CELL	Moderate	Heavy	
	Chronic	Acute	Chronic
Monocyte 	↑ Phagocytic activity ↑ IL-6, TNF-α	↓ Frequency ↓ IL-6, IL-12, TNF-α ↓ Effercytosis ↑ IL-10	↑↑↑ TNF-α
Dendritic cell 			↓ IL-12 ↑ IL-10 ↓ CD80/CD86
T lymphocyte 	↑ Frequency ↑ IL-2, IL-4, IL-10, IFN-γ ↓ IFN-γ/IL-10 ratio ↑ Vaccine responses	↑ Apoptosis	↓ Frequency ↓ Naive T cells ↑ Memory T cells ↑ Activation ↓ Antigen-specific responses
B lymphocyte 	↑ Frequency ↓ IgA, IgM, IgG	↑ Apoptosis ↑ IgA	↓ Frequency ↑↑↑ IgA, IgM

Chronic EtOH decreases host immune defenses



Alcoholic fatty liver (steatosis)

Reversible enlarged liver with lipid accumulation

EtOH impairs carbohydrate metabolism and favors fat synthesis & accumulation over oxidation & transport:

- EtOH metabolism ↑ NADH:NAD^+ ratio ⇨Lower NAD^+ levels impairs normal carbohydrate metabolism
- EtOH-induced changes in transcription factors ⇨↑enzymes for synthesis of cholesterol & fatty acids; ↓ transcription of genes involved in oxidation, transport & export of free fatty acids
- EtOH decreases metabolic regulators ⇨↓ transport fatty acids into mitochondria, ⇓ oxidation, & ↓ export of free fatty acids
- EtOH ↑ApoB degradation→↓ 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 ⇨ 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 ⇑ risk for cancer of oral cavity, throat (pharynx), larynx (voice box), esophagus and liver as well as colorectal and breast cancer

Cancer	Relative Risk
Oral & throat	5.13
esophageal	4.95
laryngeal	2.65
colorectal	1.44
breast	1.61

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)