Final Review

March-07-13
11:19 AM

A Whole Body Perspective

- Multiple organs/ tissues with specific energy requirements
    - Continuous state of flux
- What is the body's strategy to meet energy requirements (in normal circumstances)?
    - CHO (bain requires 100g/day)
        - Glycogen stoores in lover provide a daily buffer, not long-term storage
    - Protein
        - Lots of protein, but not really "stored"
        - Proteins have defines roles (enzymes, structural, etc)
        - Therefore, not typically used as an energy source
    - Fat
        - Stored in lipid droplets in adipose tissue
        - Excess lipid storage is not good in lover and muscle

Macronutrient Regulation

- Macronutrient regulation is multi-tissue
- Short-term macronutrient flux
    - Minutes to hours, i.e. between meals
    - Tissue crosstalk
        - Hormones move throughout the body and trigger signalling pathways in their target tissues
            - E.g. insulin, glucagon epinephrine, etc.
        - Usually affects protein function
            - E.g. phosphorylation or de-phosphorylation
- Long-term macronutrient flux
    - Several hours to days, i.e. fasting, starvation
    - Altered gene expression

Hormones

- Insulin
    - Secretion regulated by gut hormones -> incretins
    - Fed state
        - Insulin secretion is stimulated
    - Fasted state
        - Insulin secretion low
    - Considered "anabolic"
        - Increases glycogen stores in liver/muscle
        - Increase fat storage in adipose tissue and fatty acid biosynthesis
        - Increase protein synthesis in muscle
- Glucagon
    - Main effects in the liver
    - Maintains glucose output during fasting
- Epinephrine
    - Stimulates glycogen breakdown (via glycogen phosphorylase)
    - Stimulates lipolysis (via hormone sensitive lipase)
        - Fight or flight response

Brain

- High requirement for oxidative metabolism
- Usually glucose
    - 100-120g/day required
- Fatty acids can't cross the blood brain barrier enough o provide sufficient energy
- During an overnight fast, liver produces 2 mg glucose/ min per kg weight
    - E.g. for a 70 kg person
        - 2mg/mon glucose x 60 min/hr x 24 hour/day x 70 = 70 201g/day
    - Brain consumes half of this

Liver

- Regulating organ
    - Normal situations, liver regulates blood glucose levels
- Major site for beta-oxidation
    - Fatty acids from lipoproteins and endogenous synthesis
    - Only tissue to produce ketone bodies
        - This is key during starvation

Adipose Tissue

- Low requirement for oxidative fuel consumption
- Glucose used as energy source
    - For lipogenesis
    - FA uptake (via LPL)
- Release non-esterified fatty acids (NEFA) into circulation from lipolysis
    - Depending on circumstances, this can be a major metabolic fuel

Skeletal Muscle

- ~40% of body weight
- Nutritional status and exercise main regulators of muscle fuel consumption
- Composed of different fibres
    - Slow-twitch
        - Used for long duration, slow contraction
        - FA predominant source of energy (NEFA derived from adipose tissue)
    - Fast twitch
        - Used for short duration, quick contraction
        - Local glycogen stores provide energy

Skeletal Muscle II

- Intense Exercise - e.g. weight-lifting, sprinting
    - Anaerobic
    - Hormones too slow to act
    - Signals is Ca^{2+} release
    - Energy released from glycogen stores
    - Energy released from creatine phosphate
    - Local energy reserves used
- Sustained exercise - e.g. jogging
    - Diffusion of substrates and O_2 from blood
    - Slow process
    - Energy released from complete oxidation of glucose and fatty acids
    - Energy reserves brought to muscle
- Remember the cross-over concept
    - Low to moderate intensity exercise fatty acids are a primary

source of energy
    - Sustained high intensity exercise, glucose is a primary source of energy

Gastrointestinal Tract
- High rate of ell turn-over
- Energy required to allow for protein synthesis and production of DNA/RNA
- Small intestine
    - Glutamine main source of energy which is converted to alpha-ketoglutarate in Kreb's cycle
- Large intestine
    - VFA/SCFA from bacterial fermentation
        - In particular butyrate

After a Meal
- Dietary glucose available
- Insulin levels increase favours storage of fuels and use of dietary macronutrients

Overnight Fast
- Digestive tract empty 8 hours after the last meal
- No dietary glucose available
- Low levels of insulin
- Key points
    - Goal is to maintain blood glucose levels for the brain
    - FA can be used for energy in order to spare glucose
    - Low insulin favour adipose lipolysis of TAGs
- Key points
    - Preserve previous glucose
    - Glycogen reserves sufficient for 24 hours
    - Protein breakdown occurs initially, liberating gluconeogenesis amino acids
    - Fatty acids break down to spare protein from ketone bodies (can be used by the brain)

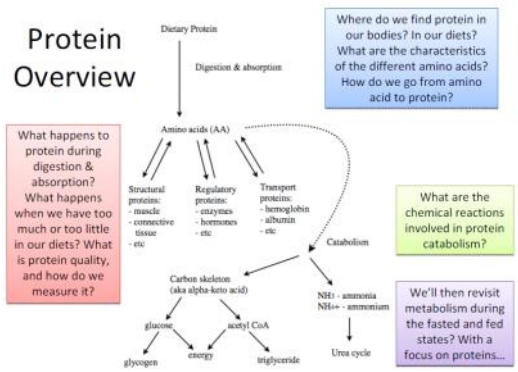
Post-Exercise Strategies
- Goals
    - Replenish muscle glycogen stores
    - Repair muscle tissue
    - Maintain hormone levels
- Catabolic to anabolic
    - The quicker the better -> liquid diet
- Liquid diet composition
    - CHO required (fast absorbing - maltodextrin)
        - Replenish glycogen & spare protein breakdown
    - Protein required (fast absorbing - whey protein)
        - Begin muscle repair
    - Fat required (fast absorbing - oil or whipping cream)
        - Maintain hormone levels (testosterone, cortisol)
        - SFA and MUFA good, not PUFA

Protein Metabolism
Macronurteints
- CHO
    - Energy source
    - Includes fibre

    - No real essential nutrients
    - Want to increase intake especially of complex CHO
    - Health Canada recommended intake is 45-65%
- Fat
    - Energy source
    - Source of n-3 and n-6 fatty acids
    - Want to decrease intake of total fat, saturated and trans fat increase n-3 intake
    - Health Canada recommended intake is 25-35%
- Protein
    - Energy source
    - Substrate for glucose synthesis
    - Provides amino acids for proteins synthesis and other areas of metabolism
    - 20 (or 21) used in making protein
        - The 21st is selenocystein, which is found in proteins but is not coded for in DNA
    - 8-10 considered dietary essentials
        - Depends on life stage (8 essential in adults and 10 in infants)
    - Protein requirement is really an amino acid requirement
        - If you are using amino acid as a protein source you are not doing well in other bodily functions
    - Average consumption (NA) about 16% of calories
    - Require about 12% of calories to meet needs
    - Health Canada recommended intake is 10-30%

Protein Overview

-



Where do we Find Protein in our Bodies

- Whole body (Reminder)
    - Water ~60%
    - CHO~0.2%
    - Fat(lipid) ~20=25%
    - Proteins~15%
    - Minerals ~2%
- Connective tissue, RBC eyes and skin have high protein content
- Adipose, mammary glands, and bone marrow are high in lipids, low in water and protein

Where do we Find Protein in our Diets

- Animal sources will provide you with all amino acids, in the amounts that you need
    - This is because animals need the amino acids too, so they will be eating what they need and will posses what they don't so when you eat them you get everything

- Not all protein we eat will give us the amino acids that we require
    - Wheat is a good example of this
- Percent protein content of animal products is generally higher than in plants

Amino Acid Structure

- Amino acid = the building blocks of proteins, they can be considered to be a monomer
- Have four components
    - The alpha carbon
        - the one that has four different side attachments
    - Carboxyl Terminal
        - Carboxylic Acid functional group contains carbonyl carbon
    - Amino Terminal
        - Amine Functional Group
    - Side chain
        - Variable composition
        - May or may not contain functional groups
        - This is the only variation that happens between amino acids

Standard vs. Non-Standard AA

- Two types of amino acids in the body
    - Standard Amino Acid
        - 22 proteinogenic AA meaning they are used to make proteins
        - 20 encoded by DNA, except:
            - Selenocystein
            - Pyrrolysine
    - Non-Standard Amino Acid
        - Usually formed by post-translational modification of other amino acids or as intermediates in the metabolic pathways of standard amino acids
        - Rarely used to make proteins
        - We won't talk about these much
        - You will not find a codon that will code for these
        - They are created by means of post-translational modification
        - They are used to make proteins but they are modifications

D vs L Amino Acids

- D vs L enantiomers
- All standard AA exist as enantiomers
    - All but glycine
- L form of AA found in nature
- D form made by posttranslational modifications

Zwitterions

- At physiological pH, amino acids are ionized
    - Protonated amino group
    - Deprotonated carboxyl group
- No overall charge (except R group)
- Increased polarity (AA are water soluble)
- Traditional presentation would occur in strong acid
- Zwitterion at physiological pH relatively polar structure

AAs Connected by Peptide Bonds

- Peptide bonds, also known as amide bonds

    - Covalent chemical bond
- Carboxyl group of one AA reacts with amino group of another AA, releasing H_2O (condensation reaction
- You can break a peptide bond by adding a water
    - A hydrolysis reaction

Protein Synthesis: From AA to Protein

- Can peptide and protein be used interchangeably?
- Amino acids are joined via condensation reactions
    - 2 Amino acids = Dipeptide
    - 3 Amino acids = Tripeptide
    - 4-50 Amino acids = Oligopeptide
    - >50 Amino acids = Polypeptide
- 1 or more polypeptide (biologically active protein)
- So it essentially turns from a linear polypeptide chain and then it is folded into a 3-D protein

Primary Structure of Protein

- Determined by the DNA sequence
- Primary structure = polypeptide of amino acids
- Amino acids held together by peptide bonds
    - Made during translations
    - They are helped along by chaperons
- Carboxyl and amino terminus, counting of amino acids starts at the amino end

Secondary Structure of Proteins

- Determined by hydrogen bonds that form a stabilized structure
    - Certain amino acids prefer one structure over the other
- Doesn't involve side chains, only backbone atoms
- Two forms of stabilized structures
    - Alpha-helixes
        - 3.6 AA/turn
        - An amino group makes a hydrogen bond with a carboxyl group 4 AA later
    - Beta-pleated sheets
        - An amino group makes a hydrogen bond with a carboxyl group in another chain or folded back chain

Tertiary Structure of Proteins

- The arrangement of secondary structures
- Involves interaction between side chains, close or far
- 1 polypeptide chain
- Disulfide bonds can form between cysteine AA
- Hydrophobic amino acids tend to be placed towards the centre of a protein

Quaternary Structure of Proteins

- Combination of polypeptides in their tertiary structure
- Not all proteins have a quaternary structure
- When proteins combine, the individual proteins are usually called subunits
- Forms a multi-subunit complex (multiple polypeptides)
    - E.g. insulin, immunoglobulins

Native vs. Denatured

- Native protein is in its normal 3D configuration

- Proteins can be denatured by
    - Heat
    - Salt treatment
    - Detergent
    - pH --> like stomach acids
- When denatures, a protein loses its bioactivity
- Denaturation affects the secondary, tertiary and quaternary structure, not the primary structure
- Egg protein for example is called albumin
    - The albumin in it's native norm is transparent and liquid
    - When cooked, the albumin becomes opaque and hard
        - This means that it is now denatured

Amino Acid Classification

- Different ways to classify an amino acid
    - Essential or not
    - Basic, acidic or neutral
    - Polar or non-polar
- You don't need to memorize the structures but you do need to know essential vs non-essential, polar vs non-polar and acidic vs basic
- From a functional perspective we need to be able to understand what properties the different amino acids will confer to a protein
    - For example, polarity, lipid solubility, sites for DNA binding, sites for phosphorylation

Essentiality

- Essential Amino Acid (Indispensable)
    - Can't be made bt the body or can't be made quickly enough to meet body needs
    - The nine amino acids are
        - Lys, Thr, Iso, Leu, Met, Phe, Try, Val and His
- Conditionally indispensable
    - Not normally required in the diet
        - Phenylketonuria (unable to breakdown Phe- a build up leads to mental retardation),, so Tyr most be supplemented in the diet
- Acquired Indispensable
    - May become indispensible in states of metabolic disorder or stress
        - Liver disease (cirrhosis) impairs Phe and Met catabolism
        - Tyr and Cys synthesized from Phe and Met catabolism, respectively
        - Tyr and Cys become indispensable in this circumstance
- Non-Essential (Completely Dispensable)
    - Can be synthesized in the body and are not essential components of the diet

Classification: Basic Amino Acids

- Basic amino acids (positive charge in amine group on side chain --> allows DNA binding)
- Basic amino acids are polar
- Important part of histones which bind DNA
- Examples are:
    - Lysine
        - Essential
        - Simple straight chain
        - Absent from grain products
        - Lysine Contingency

    - Arginine
        - Non-essential in adults
        - Synthesized during urea cycle
    - Histidine
        - Ring structure
        - Used to produce distamine (inflammation)
        - Essential in children

Classification: Acidic and Neutral Amide

- Acidic amino acids (negative charde on side chain carboxyl group)
- Acidic amino acids and neutral amides are polar
- Acidic amino acids are:
    - Aspartate
        - Non-essential
        - Involved in protein catabolism
        - Transaminated to oxaloacetate (Krebs)
        - Delivers N to urea cycle
    - Glutamate
        - Non-essential
        - Transaminated to alpha-ketoglutarate (Krebs)
        - Source of NH_3
        - Used to produce GABA (neurotransmitter)
- Neutral Amides are
    - Aparagnine
        - Non-essential
    - Glutamine
        - Non-essential
        - Important in protein metabolism because it is a carrier of N (to lover and kidney)

Classification: Neutral

- Neutral amino acids have no charge on the side chain
- They are non-polar
- Both aliphatic (C and H joined in straight or branched chains, non-ring structures)
- The neutral amino acids are:
    - Glycine
        - Non-essential
        - Used primarily to produce porphorin (component of the heme protein in hemoglobin)
    - Alanine
        - Non-essential
        - Simialr to glutamine (a carrier of N between tissues)

Classification: Branched Chain

- Also neutral aliphatic amino acids no charge on side chain)
- They are non polar
    - Val<Leu< Ile with regards to hydrophobicity
- All are branched, examples of which are:
    - Leucine
    - Isoleucine
    - Valine
- They are all non-essential
- Not catabolized in the liber, so high levels in circulation
    - They bypass the liver
- Triggers protein synthesis (protein supplements)
    - You can think of these as being anabolic, which means they

promote making protein and stuff

Classification: Hydroxylated

- Contain a hydroxyl group, which is a site of phosphorylation
- Botha re polar (because of electronegativity differences between O and H
- Tyrosine can also be classified as "hydroxylated", but it's grouped under aromatic
    - Turns on or turns off proteins by phosphorylating serine or lysine
- The two hydroxylated are
    - Serine
    - Theronine
- Serine is non-essential and threonine is essential
- -OH group is a site of phosphorylation
    - This is where a protein is turned on or off

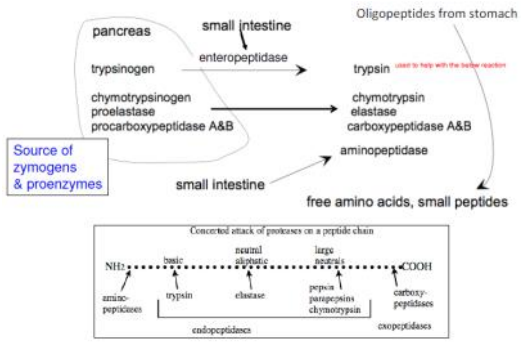
Classification : Sulfur-Containing

- Contains a sulfur group - very important functions
- Both are non-polar
- The two amino acids are:
    - Cysteine
        - Non-essential
        - Made from methionine - so if you have a diet that is poor in methionine then you would have low amounts of cysteine, this is an example in which something can become conditionally dependant essential
        - "Spares" methionine - If you have a lot of cystine you don't need to make it from methionine
        - Used to form disulfide bonds (crucial role in protein structure)
        - Used for glutathione synthesis (oxidant defense) - antioxidant properties
    - Methionine
        - Essential
        - 1st step in the synthesis of all proteins
        - Methionine is limiting in legumes

Classification: Aromatic

- All contain aromatic rings
- All non-polar except tyrosine (this is because of the hydroxyl group)
- The aromatic amino acids are:
    - Phenylalanine
        - Essential
        - Phenylketonuria (PKU) --> build-up is toxic
        - This can lead to mental retardation
    - Tyrosine
        - Non-essential
        - "spares" Phe
        - Used to synthesize neurotransmitters
    - Tryptophan
        - Essential
        - Used to make serotonin (mood)
        - Used to make niacin (Vit B3)
    - Proline
        - Non-essential
        - Important in collagen (extraceullular matrix)
        - Matrix is an extremely important aspect of tissue

Protein Digestion

- Mouth
  - No major digestion
  - Mechanical forces
- Stomach
  - HCL in gastric juice
  - Pepsin (endopeptidase)
  - HCl secreting from parital cells; triggered by gastrin, acetylcholine, histamine, etc.
    - HCl has two functions
      - Denature proteins (inactivation)
      - Activate pepsin
  - Pepsin begins to break-down polypeptide chain
    - Secreted as pepsinogen (zymogen, inactive)
      - Endopeptidate (breaks peptide bonds in polypeptide)
      - Function at an acidic pH
      - Creates large polypeptides, oligopeptides, and free amino acids
- Pancreas
  - The juice is a rich source of important digestive enzymes but they are inative until they get into the small intestine
  - Pancreative juice containing zymogens (digestive proenzymes)
- Small intestine
  - Break-down of peptides
  - Absorption
- 

Amino Acid Absorption

- Most amino acids absorbed in proximal small intestine
- Two wars amino acids are abrobed
  - Facilitate diffusion
    - This is how it starts, once the concentration starts it will switch over to an active process
  - Active transport (most common)
    - Sodium-dependent transporters (indirect ATP requirement)
    - About 60% will come in through the active transport
- Essential amino acid absorbed faster than non-essential amino acid
  - We don't know why the transporter can differentiate between the two
- Competition between amino acid for absorption exists
- Free amino acids have no absorptive advantages (protein supplementation)
  - The digested amino acids are taken up more readily for some unknown reason of the world

Amino Acid Used in Small Intestine

- Amino acids are either transported out of the intestinal cell, or used

withing the cell for
1. Source of energy
2. Synthesis of new protein

- Estimates indicate 30-40% of essential amino acid used in the small inestine
- For example, glutamine in the intestine is degraded to:
1. Source of energy
2. Stimulates cell proliferation (replaces enterocytes)
3. Prevents bacterial translocation
4. Increases synthesis of heat shock proteins (chaperones)
5. Used fro mucus production

Amino Acid Absorption

- Efficient for clearing up the amino acid from the blood
- Within the liber a few things take place
  - Used for synthesis or used for metabolism
- Liver
  - Effective clearance (50-65%)
    - 20% is used for synthesis
    - 80% is metabolized
  - First pass is metabolism
  - Not BCAA signals for extrahepatic tissues like muscle
    - Anabolic signals
      - The branched chain amino acids are not taken up by the liver, nobody knows how the liver can differenciate
  - Free Amino Acids
    - Liver tissue protein
    - Liver enzymes
    - Albumin and other transport proteins
    - Peptide hormones
    - BCAAs slip through to systemic circulation
  - Excess amino acids are degraded
  - NH_3 going to urea cycle
  - Carbon skeletons to energy
  - Lipogenesis or gluconeogenesis
- Small intetine
  - Free amino acids
  - Di- and tripeptieds
  - Some large protein fragments

Protein Quality

- 4 components to consider
  - AA composition
    - Any protein that provides all essential AA is considered "high quality"
    - Animal protein > plant protein, for example, grains are limiting in lysine, legumes are limiting in sulfur-containing AA (met)
  - Digestibility
    - Some proteins are more digestible than others
    - More digestible means higher quality
    - Animal protein> plan protein
    - Some materials, like hair, have a good amino acid balance but are indigestible
  - Presence of Toxic Factors
    - Less toxic factors means higher quality

        - Animal protein > plant protein
        - Plants contain thousands of phytochemicals, for example, trypsin inhibitors in soybeans interfere with trpsin, preventing digestion of protein
    - Species consuming the protein
        - Humans, pigs and chickens have similar protein utilization
        - Ruminants have bacteria in rumen that can make all amino acids so none are considered essential (remember than ruminants can use low quality protein sources?)

Assessing Protein Quality

1. Protein Efficiency Ratio (PER)
    - Official method in Canada for evaluation of protein quality
    - In this method, for a period of 4 weeks, young rats are fed a diet with all nutrients at adequate levels except for protein which is included at 10% of the diet (DM bases)
    - This is a marginal intake of protein, and if there is anything wrong with the protein source, growth will be limited
    - The rats are weighed at the beginning and end, food consumption is carefully monitored
    - PER = $\frac{\text{Gain in body mass (g)}}{\text{Protein intake (g)}}$
    - At 10% "perfect protein" you get 2g of rat growth
    - Pros:
        - Simple
        - Cheap
        - Very sensitive to amino acid balance digestibility, toxic factors
    - Cons:
        - Rats are not humans
        - Growth, not maintenance
        - Don't know WHY a protein is poor
2. Chemical Scores (CS)
    - The test proteins is chemically digested into free amino acids
    - These are then quantifies by chromatography, and mathematically compared to the composition of whole egg protein (reference)
    - CS = $\frac{\text{Abundance of first limiting AA in test protein}}{\text{Abundance of same AA in whole egg}}$ x 100
    - Pros:
        - Simple
        - Cheap
        - Allows for calculations to optimize mixtures with other proteins
    - Cons:
        - Doesn't account for digestibility or toxins
        - Is whole egg an ideal protein?

Nitrogen Balance

- Also known as protein balance
- Nitrogen balance is a measure of N output (urine, feces, sweat) from N input diet)
    - Nitrogen Balance (NB) = Nitrogen Intake - Nitrogen Loss
- During growth, pregnancy and times of tissue repair (NB > 0)
- Not enough protein, NB < 0
    - Problem exasperated with poor protein quality because body proteins used to generate essential amino acids
- For most adults NB = 0
- Problems with poor protein quality can be overcome by high protein

quality (developed countries)
- However, problems occur when protein quality and quantity are both low
- Protein requirements do vary with life stages
    - Higher requirements in
        - Infancy
        - Childhood
        - Teenagers
        - Pregnancy
        - Lactation
- Recommendations for protein requirements based on ANIMAL sources of protein
- Plant sources may be less digestible due to differences in the nature of protein and other components (fiber)
    - If these recommendations used plant sources of protein, the values would be higher

Abnormal Protein Intake
- What happens when you consume too much or too little of a nutrient?
    - Excessive Intake
        - High protein diets (Atkins, South Beach)
        - Protein supplementation
    - Deficient Intake
        - Deficient in protein quantity and energy
        - Deficient in protein quantity

High Protein Diets
- High protein diets are very popular now
- Typically, high protein diets are low in carbs
- 3 common high fat diets:
    - Atkins Diet (most criticized) (C:F:P = 3:64:33)
        - Why? Because carb intake is very low while fat and protein intake is very high
        - No attention to type of carb or fat consumed
    - South Beach Diet (C:F:P = 30:40:30)
        - What type of carb is consumes? Emphasis on low GI foods.
        - Emphasis is on reducing refined carbs and protein is consistent throughout the various phases
    - The Zone Diet
        - Not really a high protein diet, rather a balanced diet

Evaluation of High Protein Diets
- All high protein diets NOT created equal
    - Huge differences in macronutrient content and the types of carbs/fats consumes
    - Hard to compare the diets
- No LONG term (less than a year) studies of high proteins diets
    - Wight loss, or other marker of health?
- What we do know
    - Short term weight loss is comparable to other diet approaches
    - Some studies show improved inslin sensitivity with high protein as compared to high carbohydrate diets
    - Conflicting results with respect to effect on cardiovascular disease, a more moderate increase in protein appears to be cardio protective, but high protein is a concern

Protein Supplements

- Does weight lifting increase the RDI for protein (0.8p protein/kg body/ weight/ day)
- Protein supplements are widely used by athletes
- Deliver the correct balance of AA to muscle
    - However this could be delivered by any high quality proteins (eggs, meat, and fish)
- Most protein supplements deliver high levels of BCAA, very rapid

Marasmus

- Protein and energy deficiency
- Marasmus (protein calorie malnutrition)
    - Very low intake of balanced diet with around 8-10% protein (so just a bit low)
- Because everything is balance, body switches to starvation mode
    - Well organized utilization of body fuel stores allows survival, eventually leading to a complete loss of body fat which causes a wrinkled appearance to the skin
- Adults cope better than children
- Complete loss of body fat, feeling skin, uneven pigmentation

Kwashiorkor

- Pronounced "kwash-ee-or-kor"
- Diet has sufficient calories, but deficient protein
- Protein deficiency (1-2 % protein)
- Typically seen in developing countries where agriculture is key
- High carb foods (tuber cassava)
    - When child is weaned from mother's breast milk (very balanced source of nutrients) to cassava porridge (no protein or fat)
    - Lots of carbs but no protein to metabolize or transport
- Patients are characterized by a distended stomach, 'burns' on the skin and diarrhea
- Decreased plasma proteins cause edema

Marasmus and Kwashiorkor

- We've treated these two conditions as distinct, but the reality is that both typically co-exist
- Acute: Dietary challenge sets in and there is no change, mortality in 1-2 months
- Chronic: dietary challenge goes up and down, so people typically survive but are quiet ill for several months or years
- Both lead to immune dysfunction
- Intermediate forms between Marasmus and Kwashiorkor exist

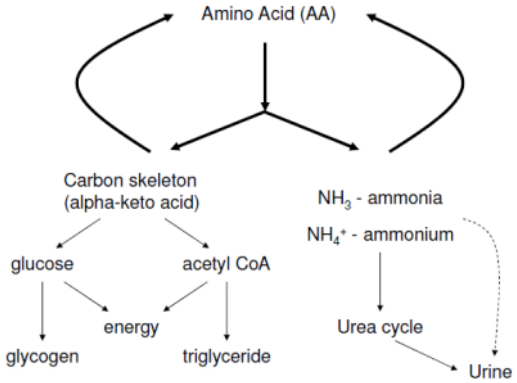
Post-Translational Modifications

- Most proteins require modification before they are biologically functional
- PTM take place in polypeptide chains, not free AA
- Phosphorylation (addition of a phosphate group) y kinases
    - Serine - OH
    - Threonine -OH
    - Tyrosine -OH
- Hydroxylation (creation of a new hydroxyl group)
    - Lysine --> hydroxylysine (very important in elastin subunits, needs copper… connection with aortic rupture)
    - Proline --> hydroxyproline (very important in collagen subunits, needs Vit C… connection with scurvy)
- Gamma-carboxylation

    - Required for calcium homeostasis and blood clotting
    - Certain proteins that are non-Ca^{2+} binding are activated to become Ca^{2+} bidning
    - Another carboxyl group added to glutamate
- Iodination
    - Critical in the formation of thyroid hormones
    - Crucial for regulation of the metabolic rate
    - Iodine deficiency in about 2 billion humans
- ADP-ribosylation
    - Adding ADP-ribose to an acceptor protein
    - Critical for DNA repair and regulation of protein function
    - Dependent on Vit B3 (niacin)
    - Niacin used to form NAD^+, when NAD^+ broken down, ADP-ribose and nicotinamide produced

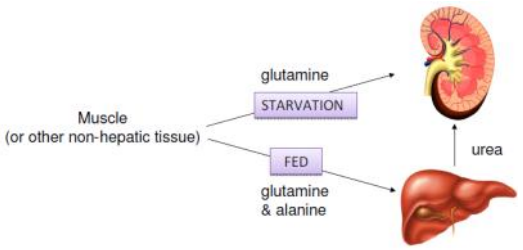
Protein Metabolism Part 2

Reactions of Protein Catabolism

- Constant turnover between protein synthesis and breakdown
- During protein breakdown --> very efficient at AA reutilization
    - ~80% AA reused
    - ~20% degraded
- 

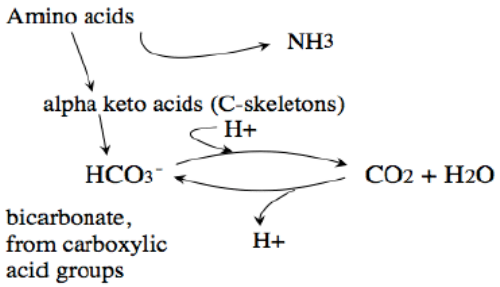
- NH_4 iss toxic, the body must buffer I, it handles it differently depending on the fed or fasted state
    - Note that the physiological state is also NH_4

Fate of NH_3 (NH_4^+) From AA Catabolism

- Three differences between fasted and fed state:
    - Fed state involves formation of both glutamine and alanine
    - Fed state involves both liver and kidneys
    - Fed state involves excretion of NH_3 as ureas, where as the fasted state involves secretion of ammonium
- 

Why the Difference Between Fed and Fast

- Metabolizing alpha-ketoacid involves production of bicarbonate (HCO^{3-})
- Bicarbonate is a weak base that reacts with H^+ (if this happens, no change in pH)
- However the fed state encourages alkalosis
- High consumption of AA causes system overload (H^+ used up and pH increases a bit (to~ 7.8)

- So why don't we die when we eat a high protein diet
    1. An active urea cycle in the fed state (mainly liver)
    2. Metabolism of sulfur-containing AA produces
- 

    - Alpha-ketoacids enter Kreb's cycle, where CO_2 produced, however at physiological pH it is actually HCO^{3-}
- In the fasted state, little protein is catabolized
- Primary source of energy --> triglycerides
- Breakdown of TG causes a production of acidic ketone bodies
- So the fasted state encourages acidosis (pH of around 7.0-7.1)
    - Also ketoacidosis
- Products of TG breakdown (long hydrocarbon chains) are not very soluble so the liver converts these long hydrocarbons into small soluble ketone bodies
- We don't die when we fact because
    - Bypass the urea cycle (uses up BO_3^-) and secrete ammonium via the kidney directly --> avoids the increase in acidic ketone bodies that happens in fed state

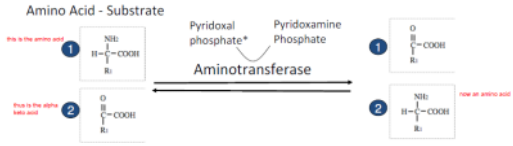
Important AA in Nitrogen Metabolism

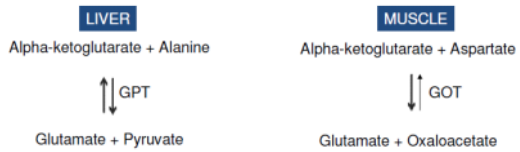
- Glutamate
    - Acidic
    - Key to metabolism
    - Source of free NH_3
    - End product of transamination reaction
        - Alpha-ketoacid = alpha ketoglutarate
        - Alpha ketogluturate Is when glutamate is broken down
- Aspartate
    - Acidic
    - Carries nitrogen into urea cycle
        - Alpha-ketoacid is ocaloacetate
        - Nb in krebs cycle
- Alanine
    - Neutral
    - Inter-organ introgen carrier (goes to liver)
        - Nb for kebs cycle
- Glutamine
    - Inter-organ nitrogen carrier (goes to liver and kidney)
    - Source of free NH_3

Four Reactions Move Nitrogen from Catabolized Protein Between Organs for Excretion

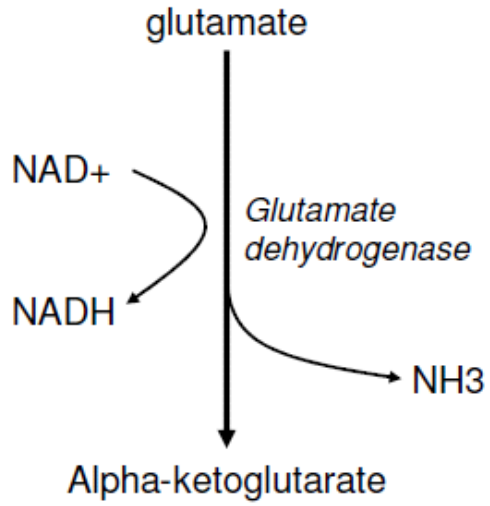
Step 1 - Transamination

- Transfer of an amino group to an AA carbon skeleton (i.e. alpha ketoacid) --> catalyzed by "aminotransferase"
- All AA undergo transamination except lysine, histidine, and threonine

-


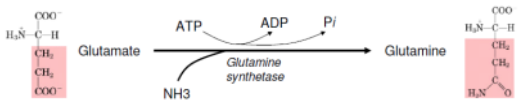
    - Essentially taking the amino group and giving it to another keto acid turning the donator into the keto acid
- Alpha-Ketoacid Product
    - Pyridoxal phosphate (active form of Vit B6) --> co enzyme that hold NH_3 group during transfer
- Bidirectional reaction
- Active in all tissues
- Always produces an AA (typically glutamate) andalpha-ketoacid
- At least 1 transaminase for each AA, with each using glutamate/ alpha-ketoglutarate as one of the pairings
    - Most abundant amino transferases:
        - Glutamate pyruvate transaminase (GTP which is also known as ALT)
        - Glutamate oxaloacetate transaminase (GOT, also known as AST)
-


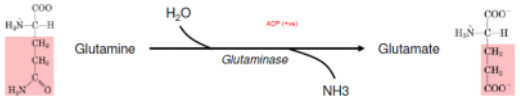
    - Glutamate and alpha-ketoglutarate play key roles in amino acid metabolism

Step Two - Oxidative Deamination
- Glutamate dehydrogenase reaction
- Glutamate is the AA to undergo oxidative deamination
- Releases free NH_3 from glutamate backbone
- Unidirectional reaction
- Process that is very active in all tissues in the body
-


- Uses of free NH_3 changes from tissue to tissue
    - In extrahepatic tissue (EHT), used for glutamine synthesis
    - In liver, used for urea synthesis
    - In kidneys, provides free NH_3 which becomes NH_4^+ and is excreted

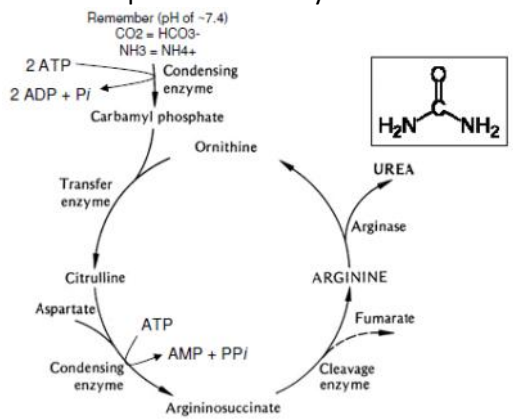
Step Three - Glutamine Production
- Formation of glutamine (major inter-organ nitrogen carrier)
- Muscle produces ~90% of the glutamine synthesized

- 

    - Glutamine in the fed state goes to the liver
    - in the fasted state goes to the kidney
    - It is and important AA for GIT
- Glutamine is most abundant in the blood
- Catabolism of glutamine, which releases NH_3 (deamination)
- In EHT, this is a process highly used by the intestine
- Active in liver in the fed state (for urea synthesis)
- Active in the kidney during fasting
- 

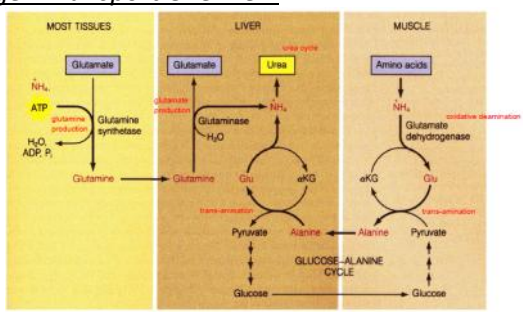
    - Bi-directional reaction with different enzymes for forward and reverse reactions
        - Form glutamine in EHT --> transport to NH_3/kidney --> remove NH_3 for excretion and regenerate glutamate

Step 4 - Urea Cycle

- Toxic NH_3 in converted to less toxic urea in the liver
- Urea transported to kidney for excretion
- 

    - Because arginine is produced by the urea cycle, it is not an essential amino acid
- Fasted state: 80-90% will be in form of NH_4^+
- Fed state: 80-90% of urinary N will be in form of urea
- NH_3 from oxidative deamination glutamine breakdown
- Aspartate produced by transamination with glutamate (produces oxaloacetate)
- Urea cycle uses up one bicarbonate, prevents alkalosis
- Urea cycle creates arginine (which is anon-essential AA)
- Urea cycle uses 4 high energy phosphate bonds
- Defects in any of the enzymes in the urea cycle leads to developmental neurotoxicity

<u>Nitrogen Transport Overview</u>

- 

    - Make better notes on this by hand when you get the chance

Summary

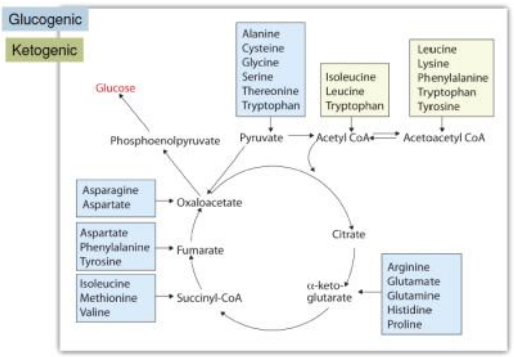
- In the fed state, EHT protein catabolism leads to glutamine formation (from glutamate) and alanine (from pyruvate). Both are transported to the liver. In the liver, glutamine and alanine will be used to form urea. Urea is then transported to the kidney and excreted in the urine
- In the fasted state, EHT protein catabolism leads to glutamine formation (from glutamate). Glutamine is transported to the kidney. In the kidney, glutamine is converted glutamate and the removed NH_3 is excreted in the urine as NH_4
    - Note that in both the fed and fasted states, while nitrogen excretion as urea and NH_4^+ dominate, respectively, there will still be a small amount of the other being excreted
    - In other words, not only one or only the other, they both occur

What About the Alpha-ketoacid

- When body's energy sources are low protein is broken down
- AA catabolism forms: Ammonia (NH_3) and Alpha-ketoacid
- Alpha-ketoacids can be formed by
    - Deamination - removal of amino group from AA, the carbon skeleton that remains is the alpha-ketooacid (mostly seen with glutamate)
    - Transamination - transfer of an amino group from an AA to an alpha-ketoacid, in the process, the AA becomes an alpha-ketoacid and the alpha-ketoacid becomes an AA
- Alpha-ketoacids contain ketone and carboxylic acid functional groups
    - Ketogeneic --> an AA degrading into Acetyl CoA by ketogenesis
    - Glucogenic --> A degraded AA that can be converted into glucose

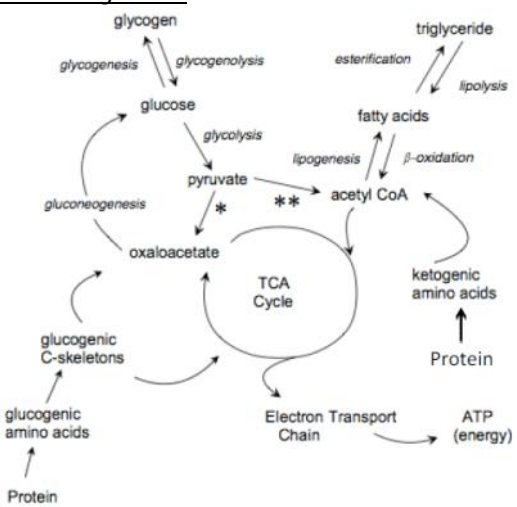
Glucogenic vs. Ketogenic AA

-



Metabolic Integration

-



    - Once you form Acetyl CoA you can no longer use the building

blocks to make glucose (purely ketogenic)
    - This is because it only has 2-C which is not big enough o make glucose
  - Intermediates with 3-6 carbons can be used to make glucose
    - Like pyruvate and oxaloacetate
  - The * means pyruvate carboxylase
  - The ** means pyruvate dehydrogenase

Fat Burns in the Flame of CHO

- Means that you need carbohydrate 'fireplace' in order to burn fat 'logs'
- To burn fat, you must have an active TCA cycle, which depends on availability of oxaloacetate
- This is not a problem while glycogen is present
- When glycogen is depleted, you have gluconeogenesis to maintain blood glucose
- Oxaloacetate is a gluconeogenic precursor, so if something else isn't doing this job, oxaloacetate will get used up and the TCA cycle slows and can't burn fat

Metabolic Integration

- Must maintain
  1. Blood glucose between 60-100mg/dL (<60mg/dL you develop a coma and die)
  2. Blood pH near neutrality (controlled by NH_3 production)
- Blood glucose is
  1. Always required by RBC as energy substrate (no mitochondria)
  2. Required by central nervous system (although gradual adaptation to ketones)
  3. Maintain Kreb's cycle
-

Metabolic State	Predominant Source of Blood Glucose
Fed	Dietary CHO (if high carbohydrate diet) Dietary protein (if high protein diet)
Post-absorptive	Glycogen from liver (direct) and muscle (indirect)
Fasting	Gluconeogenesis from protein catabolism
Starvation	Gluconeogenesis from glycerol produced by fat breakdown

Hormonal Regulation of Metabolic Integration

-

Hormone	Nature of hormone	Produced by	Effects
Insulin	Anabolic	β-cells (pancreas)	↑ glucose and AA uptake in muscle and liver ↑ glycogen and protein synthesis in muscle and liver; & fat synthesis and storage
Glucagon	Catabolic	α-cells (pancreas)	↑ breakdown of glycogen, protein & fat ↑ gluconeogenesis from AA and glycerol
Corticosteroids (cortisol)	Catabolic	Adrenal cortex	↑ Muscle protein breakdown (release AA) ↑ gluconeogenesis from AA
Catecholamines (epinephrine)	Catabolic	Adrenal medulla	↑ glycogenolysis and **lipolysis**

- T3 (T4) - tyrosine-based hormones produced by thyroid that affect metabolic rate

Fed State

- Last 0-2 hours
  - Food in stomach and GI tract
- High blood glucose and gluconeogenic AA
- Insulin secreted
- Glucose uptake and glycogen formation induced
  - First in liver, then in muscle
- AA catabolized by liver (first pass metabolism)
- BCAA go to muscle and promote protein synthesis
- Fatty acid storage in adipose

- Alanine and glutamine carry nitrogen
    - Urea produced because of alkalosis conditions (pH)
- 90% urea and 10% NH_4

Post- Absorptive State

- Gut is empty of food
- Decrease in blood glucose
    - Increase in glucagon
    - Increase in glycogen breakdown
        - Muscle secretes lactose (Cori cycle)
        - Liver secretes glucose
- Necessary to maintain glucose for RBC and brain
- Blood glucose supports Kreb's cycle in all tissues
- Alanine and glutamine carry nitrogen
- Fat not yet used as a source of energy
- Muscles make lactose to go to liver and cor cycle

Fasted State

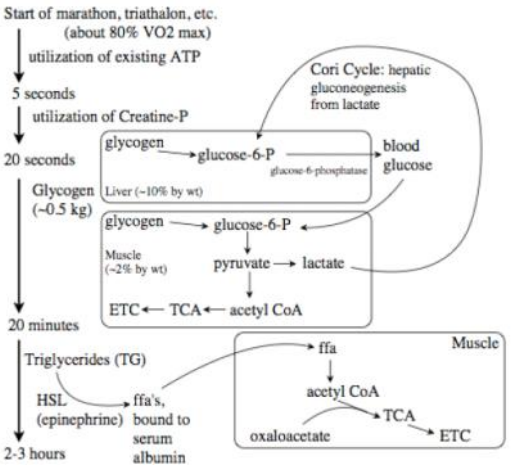
- Happens more than 12 hours after a meal
- Glycogen pools empty
- Corticosteroids secreted from adrenal gland to promote protein catabolism
    - Mostly in muscle, but also GIT
- Glucogenic AA used to produce glucose in liver
    - Important for RBC, brain and Krebs
- RBC and brain still required glucose for energy
- Blood glucose supports Kreb's cycle in all tissues
- Alanine and glutamine carry nitrogen
    - More of a mix between urea and ammonia
- Fat starts to be used as energy

Starvation State

- Fasted state not sustainable
    - Need to switch to fat
- Corticosteroids and thyroid hormones no longer secreted, but glucagon is
    - Spares protein loss
- Fat is predominantly ketogenic (only glycerol backbone is glucogenic)
- Tissue metabolism adapts
- Liver produces ketones (4 carbon organic acids that are soluble)
- Promotes acidosis
- Still have to maintain TCA cycle
- Glutamine carries nitrogen directly to kidney
- Leads to marasmus
- Conserve bicarbonate to counteract acidosis
    - Decrease urea cycle
    - Secretes NH_4^+ into kidney

Exercise (Sustained Aerobic)

-



  - Progressive mobilization of energy stores to maintain blood glucose and energy
  - Eventually lead to exhaustion which will be due to loss of glycogen pools leading to a drop in blood glucose or failure of tissue TCA cycle activity (drop in oxaloacetate)
  - Epinephrine starts lipolysis is breakdown fat for E which is sustained
  - This cannot run forever even with an insane amount of fat because glycogen will deplete --> no glucose --> no krebs--> no fat

How to Extend your Exercise Capacity

- Carbohydrate loading while resting
  - Can double muscle glycogen
  - Delays failure of the TCA cycle
- Fasting for 3 hours prior to start
  - Avoid insulin secretion
- Caffeine
  - Earlier release of epinephrine, which activates HSL
    - The release of epinephrine mobilizes fatty acids before you start7

Micronutrients Part 1

Vitamins and Minerals: The Basics

- Characteristics for classifications as vitamins:
  - Exogenous supply is required
    - Foods or indirectly from the gut, outside the body
  - Needed in small amounts
  - Distinct from sugars, fats and proteins with regards to structure and function
  - Performs at leas one essential biochemical function
  - When lacking n the diet, a characteristic deficiency disease develops
  - Organic
    - Main distinction from minerals (which are inorganic)

Ways to Discuss Micronutrients

- Traditional discussion
  - Fat soluble vitamins, water soluble vitamins and minerals
- Functional groupings/ practical importance discussion
  - Group I: Micronutrients which control type II steroid hormone receptors and have major global health implications
    - Iodide, Vit A, Vit D, Clcium, Vit K, Phosphorous, Fluoride
  - Group II : Micronutrients that work together in oxidant defense

        - Vit E, Selenium, Vit C, Niacin, Riboflavin, Copper, Zinc, Manganese
    - Group III: Micronutrients that act as enzyme cofactors
        - Thiamin, Niacin, Riboflavin, VitB6, Folate, VitB12, Biotin, Pantothenic acid
    - Group IV
        - Iron, copper, and zinc-related divalent cations
- 1 out of 3 people in developing countries are affecting by vitamin and mineral deficiencies

Group 1

- These micronutrients control cellular function through a certain type of interaction (type II steroid receptors)
- Not all act necessarily on steroid hormone (only Vit A, Vit D, and iodine)
    - But, you can't talk about Vit D without talking about calcium, phosphorus and Vit K, which are all involved in bone metabolism
- Vit A consumed as retinol or beta carotene (which is not biologically useful to us), which is then turned into retinoic acid or ratinal, which is a marker
- Vit D precursors converted to calcitrol, which regulates Calcium levels in the body
- Iodine used to make hormone T3 and T4 which regulates synthesis of proteins that control BMR
    - Iodine is converted into hormones which is important in metabolism

Steroid Hormone Receptors

- Steroid Hormone Receptors --> needs ligands to activate it
    - Intracellular protein receptors that bind to hormones and become active transcription factors
- Type 1 Receptors --> Cytosolic
    - Respond to steroid hormones like estrogen, testosterone, progesterone glucocorticoids, and mineralocorticoids
- Type 2 Receptors --> Nuclear
    - Respond to steroid and non-steroid ligans, like thyroid hormone, retinoic acid, and calcitrol
    - This type will affect gene expression

Iodine

Iodine in the Diet

- Iodine (inorganic material) content of most foods is low
- Iodine found in higher concentrations in coastal populations compared to mountains regions
    - Seafood has high concentrations (especially sea greens)
    - Mountainous regions are less so because water washes it away easily

Iodine Absorption

- Iodine is typically found in the body in its ionic form (I^-)
    - 70-80% is located in thyroid gland
- Dietary iodine can be bound to AA or found free
- Rapidly converted to I^- and absorbed
    - Mostly in the stomach (90-95%), a bit in the small intestine
- In the blood, free I^- is found and can permeate all tissues (mostly thyroid gland)
- Active system in thyroid (Na^+/I^- symporter

Iodide Transport and Functions

- All tissues depend on thyroid hormones (T3 and T4) rather than iodide
- Once T3 and T4 made, they are secreted into blood and transported by specific carrier proteins (albumin transthyretin, etc)
- 50x nire T4 in plasma compared to T3, but T3 is 100x more potent
    - T3 interacts with thyroid hormone receptor (THR)
    - T3 and T4 production regulated by TSH
- Steps
    1. I^- is absorbed by means of GIT and transferred to the blood
        - This happens by means of a Sodium Iodine symporter which is indirectly dependant on ATP
    2. I^- is then transferred to the tyroid by means of an active transporter
        - The thyroid is responsible for both storage and secretion of T3 and T4
        - Thryoglobulin (THG) is the protein that produces T3 and T4 when iodinated
    3. T4 then goes to the liver where an enzyme removes I^- and 5'-deiodinase (selenoprotein) changes it to T3
        - T3 regulates metabolic rate and growth in many tissues through interaction with the thyroid hormone receptor (THR)
    4. If T3 is low, the hypthalamus senses it, and neural signals to the pituitary cause release of TSH
    5. TSH (thryoid stimulating hormone) will then go to thyroid and promote the secretion of T4/T3

How Does Iodine Make Thyroxine (T4)

- In the colloid, iodine is oxidized to form a free radical
- Thyroglobulin (TG) produced in thryod gland
    - Tyr-rich protein
- Iodine radical iodates the Tyr residues and crosslinks tyrosines to create hormones (eventually lots of T4 and T3)
- Still bound to THG, so in the thyroid cell protease hydrolyze THG, releasing T4 and T3

Thyroid Hormone Signalling

- T3 and T4 are lipophilic (can cross plasma membrane easily)
- In liver, T4 converted to T3 which binds to THR on DNA during signaling
- In many tissues, T3 binds Thyroid Hormone Receptors (THRs) and bind to response elements in promoter regions of DNA --> this leads to the formation of new mRNA for specific proteins
    - It can also stimulate transcription
- The induced proteins have wide ranging effects, leading to increased metabolic rate and play a key role in fetal development
    - ATPases (pump Na^+ and Ca^{2+} out of cells), which increases metabolic rate
        - Na^+ - muscle contraction, neuron firing
        - Ca^{2+} - signalling events
    - Growth hormone - anabolic effects

Iodine Deficiency

- When T3 levels are low, the pituitary gland releases TSH
    - T3 is marker for TSH release
- If iodine deficient, thyroid still being stimulated to produce hormones, but can't
    - Hyperplasia (increase in cell number)

    - Hypertrophy (increases cell size)
- The result
    - Goiter --> Thyroid enlargement
        - If left untreated, comes thryoid cancer
    - Cretinism --> when iodine levels are low in the mother, the fetus doesn't develop properly
        - Irreversible
        - 50 million people have some degree of mental impairment caused by IDD
- Growth and developmental abnormalities
- Outcomes include (going from mild to severe deficiency)
    - Nodular goiter and hyperthyroidism, lower intelligence, poor educability
    - Increased infant mortality, Goiter and hypothyroidism
    - Mental retardation cretinism, poor growth and stunting

Vitamin A

- Night blindness was a recognized diseases before 1500 BC
- The alcohol form retinol (animal based) and the carotenes (plant based) were recognized as the plant form of vitamins
    - A plant provitamin the form of a vitamin produced by plans that when consumed by animals is converted into the bioactive vitamin
- Vitamin A is generally used to referred to a group of compounds retinoids that have the biological activity of all trans-retinol
    - Retinol, retinal, and retinoic acid
- Carotenoids (provitamin A) are precursors for Vitamin A
    - Pigments produced in plants like beta carotene, alpha carotene, etc

Digestion and Absorption

- Retinyl esters (milk and eggs)
    - Retinol and fatty acid 16:0
    - Retinyl esterase remove fatty acid
- Beta-carotene (plants)
    - Passively absorbed
- Lipophilic (mixed micelles)
- Both absorbed by passive diffusion
- Beta-carotene has two fates
    - Converted to retinyl ester, VitA
    - Incorporated into chylomicron which is used for transport and storage
- Everything goes to the liver in CR
    - Beta-carotene gets packaged into VLDL and stored in adipose
    - Retinyl esters stored in liver (usually as retinyl palmitate) n stellate cells
- 70-80% of dietary vitamin A is absorbed
    - 10-50% of beta carotene is absorbed
    - The presence of fibre will lead to a decrease of carotene absorption
- Beta-carotene and retinol both get dumped in the liver
    - When there is a lot of Beta-carotene store in adipose tissue there is an increase in pigmentation so you turn orange
    - When there are low level of retinol-RBP it stimulates retinyl esterase to release retinol from storage
- Key points of vitamin A digestion and absorption are
    - Vitamin A and beta carotene are lipophilic
        - Therefore they are treated like other dietary fat

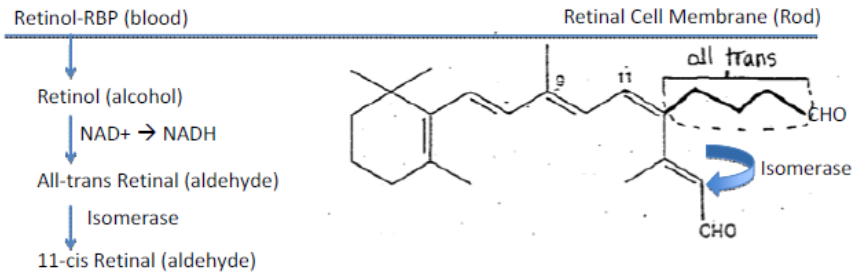
- Depending on the Vit A status (which is very stable in normal situations) of the person, beta-carotene will be
  - Incorporated into chylomicron directly
  - Converted to retinyl palmitate and hen incorporated into chylomicron
- Take up predominantly by the liver, where
  - Beta-carotene is shipped to the adipose tissue in VLDL
  - Retinyl palmitate is stores in stellate cells until needed
    - When needed, liver retinyl esterase cleaves the FA freeing retinol, which binds to retinol binding protein (RBP) and released into blood
    - RBP synthesis depends on vitamin A status, but also zinc and protein

Important Molecules

- In the intestine
  - Primary provitamin A = beta-carotene
  - Conversion to retinyl ester
  - 15,15' - carotenoid monooxygenase
    - Located in duodenal mucosa
  - Retinal (aldehyde)
  - Retinol (alcohol)
    - Acts as a detergent which is why it is converted into a retinol ester
    - No function

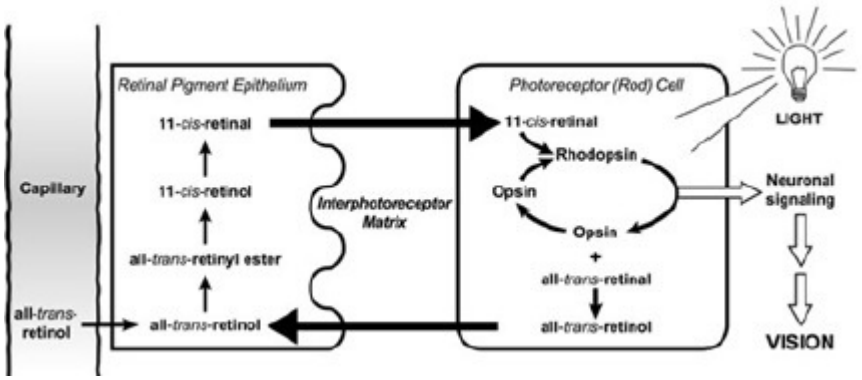
Vitamin A and Night Blindness

-



  - Rods use rhodopsin to absorb grey light
  - They have a greyish purple color
  - This is because of retinol
  - Opsin + 11-cis-retinal is very sensitive to light
  - Light breaks the bond

-

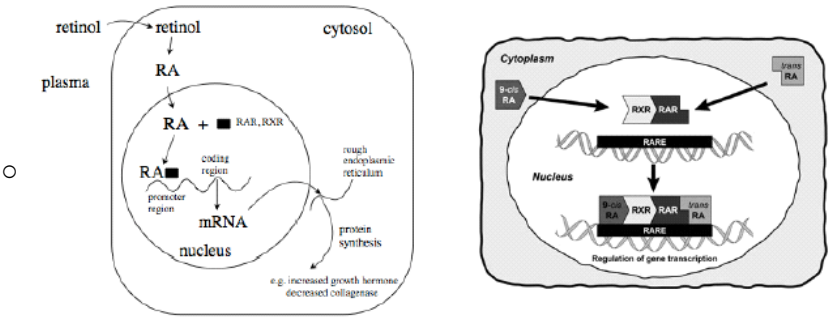


Retinoic Acid (RA) Signalling

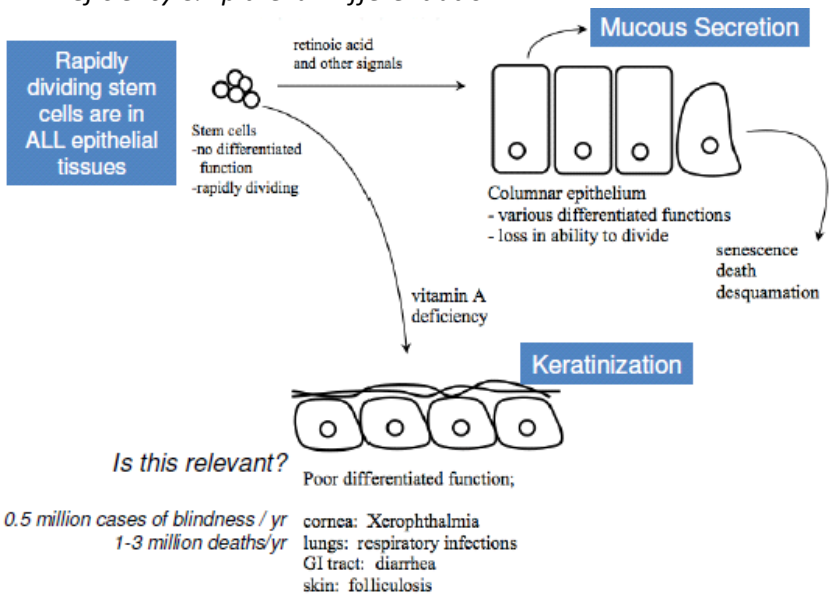
- Retionl-RBP brings retinol to a cell, which is then converted to RA
- RA goes to the nucleus where it binds and activates both the retinoic acid recepyor (RAR) and the retinoid X-receptor (RXR) families
- These complexes homo/heterodmerize with other NHR, creating a huge

number of possible variations, which allow fine variation in gene expression

- Produces growth hormone --> important for growing and differentiating tissues



Vitamin A Deficiency & Epithelial Differentiation



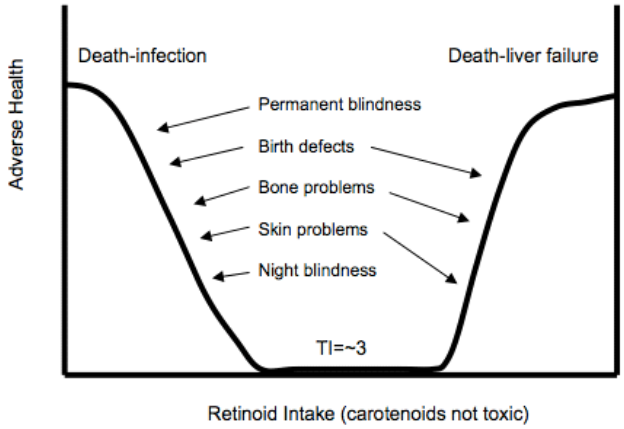
Consequences of Vitamin A Deficiency

1. Night blindness (rhodopsin)
    - Low retinol --> low ratinal --> no rhosoplsim
    - Reversible, one of the first signs of vitamin A deficiency
    - Associated with Bitot's spots, a buildup of keratin debris in the conjunctiva of the eye
2. Impaired epithelial cell differentiation
    - Low retinol --> low RA --> low mucus
    - Can cause permanent blindness and life threatening infections
3. Impaired growth (growth hormone not produced)
    - Especially of long bones and tooth decay
4. Impaired fertility
    - Decreased sperm formation, fetal resorption (early death of embryo)
5. Fetal development defects
    - Birth defects due to loss of control of differentiation
    - Can occur with too little OR too much vitamin A

Vitamin A Dietary Requirements

- RAE accounts for differences in biological activity of carotenoids
- RDA for adults :
    - Men --> 900 ug RAE
    - Women --> 700 ug RAE
- UL = 3000 ug/d retinol

- Why no UL for B-carotene
    - Stored in fat tissue only converted into retinol when in need
    - Only problem is that you turn orange
-



Vitamin A Dietary Toxicity

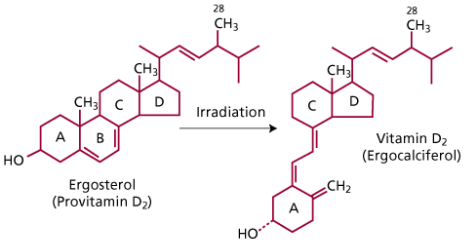
- Most severe consequence is liver cell death
- Retinyl palmitate is stored in the stellate cells of the liver and with excess vitamin A intake, the cell get full to capacity
- Raw vitamin A spills out and the local hepatocytes become damaged and die
    - Death can result from liver failure
- Excessive intake of B-carotene is not toxic, but can cause hypercarotenosis
- Accutane, and acne drug that contains 13-cis retinoic acid, can cause birth defects in women in the early months of pregnancy

<u>Vitamin D</u>

- While Vitamin A and iodine are deficiencies of the developing world, vitamin D is the most prevalent micronutrient deficiency in the developed world
- Vitamin D acts as a hormone (true steroid hormone)
    - Made in one tissue (kidney), acts on other tissues
    - Works with other hormones such as parathyroid and calcitonin
- Evidence of rickets (Vitamin D deficiency) in skeletons from over 50 000 years ago
- Medically described in 1695
- 1919 - rickets was induced in dogs, treated with cod liver oil
    - Rediscovery of use of cad liver oil against bone disease in the 1700s
    - Sunlight also used to cure rickets
    - Suggested fat soluble vitamin D could also be synthesized in the body by sunlight

Vitamin D Sources

1. Natural plant sources
    - Provitamin D2
    - Shitake mushrooms
    - Not very active in plants so not a rich source of vitamin D

- 

2. Natural animal sources
    - Provitamin D3
    - Not many, fish, fish liver oils
    - 7-dehydrocholesterol converted to cholecalciferol by sunlight (UVB and infrared)
        - Cholesterol is produced in the skin
    - In sebaceous glands of skin
3. Sunlight
    - Vitamin D3 made in the skin, binds vitamin D binding proteiN (DBP)
        - Transported around the body
        - This is not an eternal source, it can and will run out, when you go into the sun youc an convert it one to the other
    - Lumisterol and tachysterol are also produced (inactive products) that are eventually lost as we shed skin cells
        - Or converted back to Provitamin D3
    - Melanin in the epidermis absorbs UV rays, limits vitamin D3 production
        - Part of the problem for population deficiencies over time (migrating populations)
4. Supplementation
    - Not thought to be necessary for bone health, but rather cancer prevention
    - Should use Vitamin D3
        - Cholecalciferol
    - Fat soluble (better absorbed)
        - Will be digested/ absorbed att the same time as the rest of the stuff we eat, so if we eat this fat soluble vitamins when you eat with ill improve the absorption of the compound
5. Fortification
    - Government initiative
    - Milk and margarine

Vitamin D From Diet and from the Sun

- Skin/Sun
    - 7-D rapidly converted to vitamin D3 (10 min)
    - Can't cause vitamin D toxicity from sun
    - Enters blood bound to DBP
        - Liver (conversion)
        - Adipose (storage)
- Diet
    - Absorbed passively in ileum (about 50%)
        - This is because it is lipophilic
    - Enters blood in chylomicrons
        - Taken up by liver in remnants
        - Most goes in through passive absorption

Vitamin D3 Conversion in the Liver

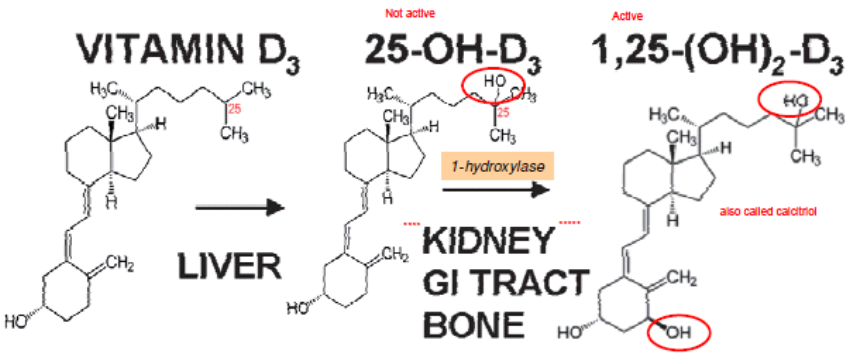
- In the liver, the 25th site on the vitamin D3 hydroxylated to from 25-OH

D by 25-hydroxylase (a cytochrome p450 enzyme)
    - NADPH- dependant
    - Occurs in the mitochondria of the liver
- This enzyme is also found in lungs, intestine, and kidneys
    - This happens predominantly in the liver
- Once produced, 25-OH D secreted into blood bound to DBP
    - Largest pool of 25-OH D in body
    - It is produced in the liver
- If there are low levels of 25-OH D, this is a key sign of vitamin D deficiency
- If calcium levels are low, this triggers conversion of inactive 25-OH D into active form
    - Produced in the kidney

Active Vitamin D

- High gradient of blood to intracellular Ca^{2+} essential
    - Drives processes that calcium has a tae in
- Low blood Ca^{2+} sensed by parathyroid gland
    - Releases PTH (parathyroid hormone)
- Promotes the uptake of 25-OH D/ DBP complex in the kdiney
    - Endocytosis
- 1-hydroxylase activates Vitamin D
    - 1-25- $(OH)_2$ D = calcitrol
        - This is the active one that targets the kidney, bone and gut
        - It's sole purpose is to make sure calcium concentration rises back up
    - 25-OH D +OH --> 1,25-$(OH)_2$ D

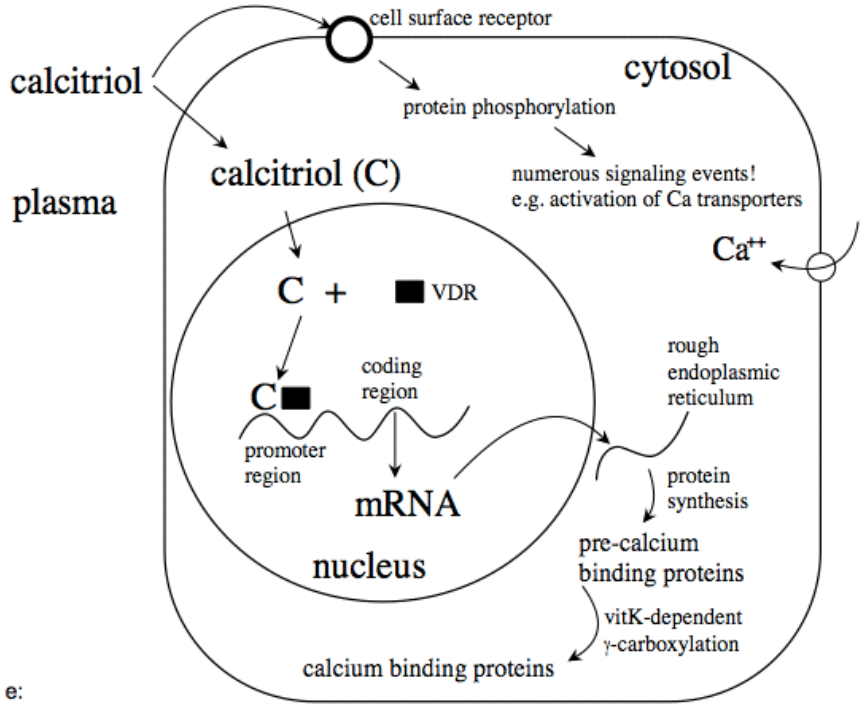
Vitamin D3 Conversion in the Kidney

-


Calcitriol Signalling

- Vitamin D activated in kidney and then sent out into the body
- 2 intracellular signalling pathways
    - Genomic
        - Vitamin D receptor (VDR)
        - VDR = NHR
        - Transcription factor promoting calcium binding protein synthesis
        - Activated by PTM
            - Vitamin K --> Cofactor
            - Gamma-carboxylation
    - Non-Genomic
        - Also binds cell surface receptors, like MARRS (Membrane-Associated Rapid Response Steroid -binding protein)
        - Trigger intracellular signalling cascades
        - Very fast, no dependence on vitamin K
- Bone uses both pathways

-



e:

- Inside the nucleus is genomic and outside the cytosol is non-genomic

Outcomes of Calcitriol Signalling

- In bone
  - Elevated calcitriol and PTH work together to stimulate resorption of Ca^{2+} and P from bone
  - Calcitril causes osteoblasts to increase expression of RANK ligan (RANKL), a cytokine
  - RANKL is secreted from osteoblasts and activates osteoclasts to increase their activity
  - Osteoclasts secrete factors that degrade the bone matrix, releasing Ca^{2+} and P into blood
- In Intestine
  - Primary function is to increase absorption and reabsorption of Ca^{2+}
    - So we don't loose any in the feces because if we lose in the feces then we did not absorb it and it was wasted
  - Genomics and Non-genomic effects
    - Calcium binding proteins are synthesized and require vitamin K dependent gamma- carboxylation
    - Membrane transporters
    - MARRS activation (non-genomic)

Organs Relevant for Vitamin D/ Ca^{2+} Story

- Absorption (small intestine)
  - Making sure you don’t loose anything to feces and important in getting Ca^{2+} from lumen to blood
  - Minerals need transporters to get through the membrane into the mucosal cells and more transporters to pass into portal circulation
  - Proper absorption depends on expression of Ca^{2+} binding proteins in epithelial cells
- Reabsorption (Kidney)
  - Turns on the various pathways that need to be turned on, so you aren't loosing anything to urine
  - Small molecules like Ca^{2+} circulate in blood and eventually reach the kidney, where they pass through the filter and can end up in

the urine unless reabsorbed, reabsorption removes the molecules from the filtrate and gets them back into the blood

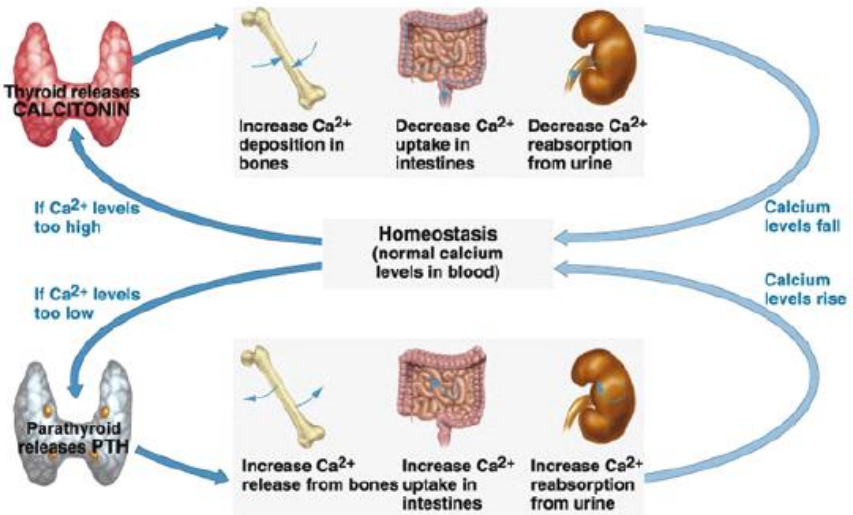
- Resorption (bone)
    - Freeing up calcium from the bone
    - Dissolving bone structure to release Ca^{2+} into the blood
        - Osteoclasts resorb, osteoblasts build
        - Balance between synthesis and resorption

Ca^{2+} Hormonal Control System

- Key point
    - Maintaining blood Ca^{2+} more important than maintaining Ca^{2+} reserves in bone (as seen with pregnancy and lactation)
    - Blood Ca^{2+} > Bone Ca^{2+}, this is why preggos are told to get a shit ton of calcium so bone degradation doesn't happen in response to supporting the feces
- Vitamin D (Calcitriol)
    - Serves to INCREASE blood calcium
        - Stimulates Ca^{2+} resorption from bone (immediate response)
        - Facilitates absorption of Ca^{2+} from intestine (short term response)
        - Maximizes tubular reabsorption of Ca^{2+} in kidney (short term response)
- PTH (Parathyroid Hormone)
    - First response because it makes calcitriol
    - Secreted by the parathyroid glands
    - Serves to increase blood calcium (1st response)
        - Stimulates production of calcitriol in kidney (activates 1-hydroxylase)
        - Stimulates resorption of bone (activation of osteoclasts)
        - Maximizes of tubular reabsorption of calcium in kidney
        - No effect on small intestinal absorption
- Calcitonin
    - Turns things off when things are back to normal, kind of ying and yang
    - Secreted by parafollicular cells in the thyroid
    - Serves to decrease blood Ca^{2+} (e.g. in response to Ca^{2+} rebound)
        - Suppresses tubular reabsorption of Ca^{2+} kidney
        - Inhibits bone resorption and facilitates remineralisation

Calcium Homeostasis

-



    - Works like a number eight and is in constant motion

Vitamin D Deficiency

- Deficiency varies across the lifespan
- Composition of normal bone
    - Mixture of solid (outer) and spongy (inner) parts
    - What is mineral and what is organic
        - Solid part --> 60% mineral (Ca^{2+}, P) and 40% organic (collagen)
        - In babies, bones start as collagen and gradually become infused with mineral
        - Deficiency concerns solid bone only
        - 60/40 ratio begins to drop --> 50/50 --> 40/60
- Infants --> Rickets (poor mineralization)
    - When you are born the bones are almost all collagen which is the soft part of the bone
    - Bones don't mineralize properly and can't support the body's weight when they start walking (permanent)
    - Was seen in Britan during industrial revolution
- Adolescents to Adult --> Osteomalacia
    - Bones become demineralized (can be reversed with supplementation)
    - Fractures can occur easily
- Middle aged to Elderly --> Osetoporosis
    - Normal part of aging (loss of both mineral and organic parts of bone)
    - Diagnosed with bone density scans
    - Difficult to reverse due to erosion of bone (holes in bone form)
- Best supplementation method? Take both Vitamin D3 and Ca^{2+}
    - This is when the case is reversible

Osteoporosis

- Osteoporosis --> bone loss association with aging
- Worsened by chronic low Ca^{2+}, Vitamin D, and Vitamin K intake
- Fractures like the hip often lead to mortality in the elderly
- Men have higher peak bone mass between 20-30 years and lower bone loss
- Treatments include supplementation or drugs that affect bone formation/resoprtion

Seasonal Variation

- An Increase in vitamin D improves bone mass; decrease colon, prostate and breast cancers; diminishes MS, psoriasis, rheumatoid arthritis; decrease hypertension and CVD; decreases diabetes improves muscle strength and motor nerve function in elderly
- Supplements can counteract seasonal variation

Recent Changes to the RDI for Vitamin D

- The RDA for vitamin D intake for adults = 600 IU (international units)
    - 1 ug vitamin D = 40 IU, so this would mean 15 ug of vitamin D/ day
        - It is so low because you can make it from the sun
        - You only need ten minutes in the sub to use up all the local stuff
- RDA recently increased (Nov 2010)- it tripled
    - Used input from research, stakeholders and scientists
- They cahnges because we found that most Canadians are deficient, especially darker skinned people

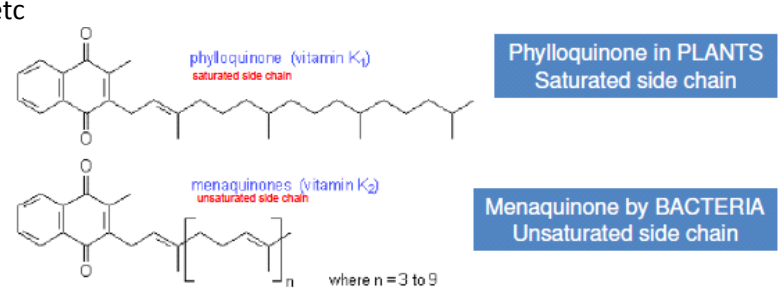
Vitamin D Toxicity

- No risk of vitamin D toxicity from the sun exposure because it is a limited source
    - Production of vitamin D3 is limited by amounts of 7-dehydrocholesterol present in skin
    - Also produces the inactive lumisterol and tachyesterol metabolites with prolonged sun exposure
- Can get much higher levels of circulating 25-OH D from dietary intake
    - Vitamin D precursors are absorbed and incorporated into chylomicrons and CR deliver vitamin D to the liver where it is hydroxylated at position 25 and returned to circulation as 25-OH D bound to DBP
    - May cause hypercalcemia, which possible calcification of soft tissues
- Circulating levels of > 500 nol/L associated with toxicity
    - This is very unlikely, and having a little bit too much for one would not do anything
- People with vitamin D intake >10 000 IU (or more)/ day for several months experience toxicity (hypercalcemia)
- This can happen because foods are fortified with vitamin D now

Vitamin K

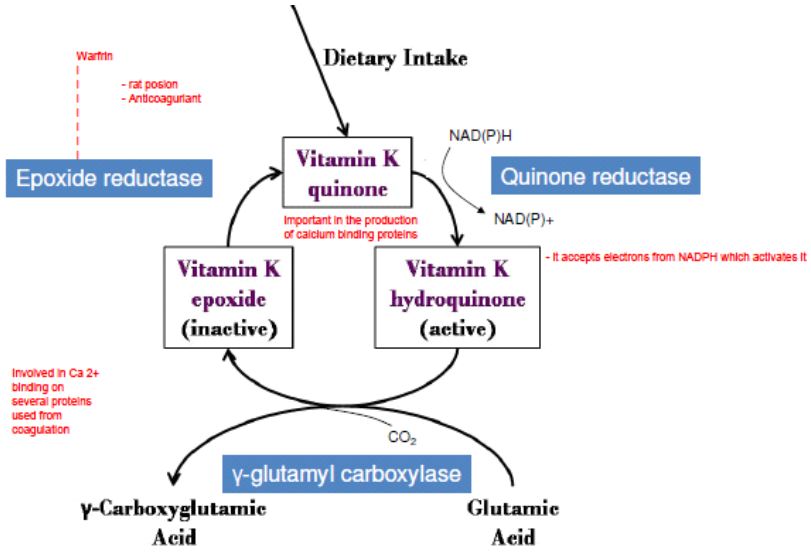
- Named after the Danish word "koagulation" which means coagulation
- Chicks fed a low-fat and cholesterol-free diet bleed excessively and their blood to a long time to clot when vitamin K added to the fed, the problems were revered
    - Dam and Dosiy won the Nobel prize in medicine in 1941 for the discovery
- Babies born with poor vitamin K status because there is little vitamin K in mother's milk
- Haven't developed microflora in gut (first 12 days) to produce vitamin K
- Provided by green leafy plants (as phylloquinone)
    - Sensitive to light and heat
- Most of vitamin K cromes from colonic bacteria, in the form of menquione
- Deficiency rare in adults due to colonic synthesis

Digestion and Absorption

- Phylloquinone requires no digestion
    - Incorporated into micelles and absorbed in the small intestine (jejunum)
    - Apical active transporter remains unidentified
- Menaquinones synthesized by bacteria in the colon
    - Passive absorption
    - Using menaquinones varies from person to person
- Both incorporated into chylomicrons and delivered to various tissues
- Vitamin K stored in cell membranes in lungs, kidneys, adrenal glands, bone, etc
    - 

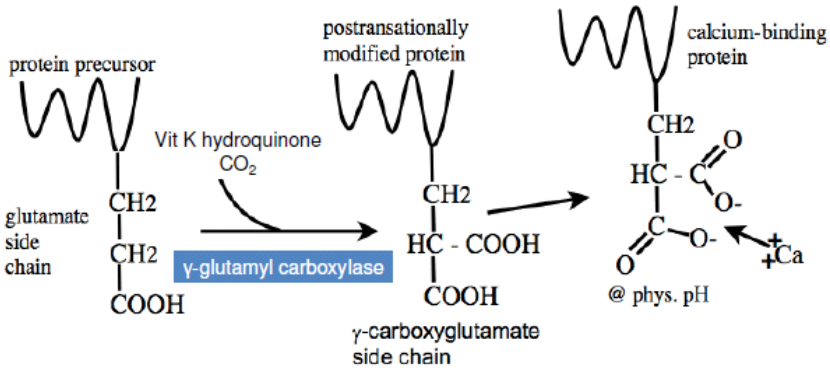
Vitamin K Cycle

-



Vitamin K and Gamma-Carboxylation

-



- Gla residues on blood clotting proteins bind Ca^{2+}, Ca^{2+} mediates binding of Gla-containing proteins to phospholipids on membranes of blood platelets and endothelial cells

Vitamin K Deficiency and Toxicity

- Virtually no instances of toxicity in adults
- Susceptible populations
  - Newborns (injected with phylloquinone after birth)
    - Heel prink to give their a gut a chance to get going
    - Not vitamin K in milk
    - Little vitamin K can cross the placenta
    - Gut bacterial population not established
  - People consuming antibiotics chronically
    - Antibiotics destroys the gut bacterial community
  - People with malabsorptive illnesses (IBD, Crohn`s pancreatitis)
- Deficiency symptoms related to role in gamma-carboxylation
  - Impaired blood clotting
    - Possible hemorrhagic syndrome (mostly newborns)
  - Impaired activation of calcium binding proteins
    - Osteoporosis
      - Remains inconclusive

<u>*Calcium*</u>

- Represents about 40% of the body`s mineral mass
  - 1000-4000 mg in human body
  - Bones and teeth contain about 99% of the calcium
  - The remaining 1% important for signalling pathways
- Predominantly from dairy products, but also high in sardines, salmone

and some green leafy vegetables
    - Present as insoluble salts
    - Stomach acidity creates soluble Ca^{2+}
- Absorption (25-30% of dietary calcium absorbed)
    - Saturable, carrier-mediated, active transport
        - In duodenum and jejunum (regulated by calcitriol)
        - 60% calcium absorbed this way
    - Diffusion via paracellular route
- Transport in body
    - 40% bound to albumin
    - Up to 10% found complexed with sulfate, phosphate, etc
    - 50% found in free (ionized) from

Functions
- Bone (99%)
    - Minerals (calcium, phosphorus, fluoride, magnesium, potassium, etc) make up hydroxyapatite (a crystal like structure)
        - This is about 60% of solid bone mass
- Intra- and extracellular (1%)
    - Ionized calcium is active (0.5%) and used for:
        - Blood clotting (via Gla residues)
        - Skeletal muscle contraction (release of calcium stores)
        - Nerve potential (via ion channels)
        - Intracellular signalling pathways
            - E.g. an increase of Ca^{2+} activates PLA_2, which cleaves AA from PL to produce eicosanoids
    - If intra-and extra-cellular levels drop, bone will be sacrificed otherwise death occurs,
        - You will die within seconds

Body Distribution of Calcium
- Intracellular Calcium
    - Mostly stored in mitochondria and ER
    - Released from stored in response to an extracellular signal (e.g. receptor binding)
    - Transduces signal into an intracellular response (gene, expression, neurotransmission, etc)
- Blood Calcium
    - Approximately 1/2 is bound to proteins
    - Maintained at a very constant level
        - About 10 000X the concentration of intracellular calcium
    - Nearly identical to the concentration of phosphorus
- Bone Calcium
    - The majority (around 99%) of the calcium in the body is in the bone and teeth
    - In the bone 99% is in mineral phase (hydroxyapatite, with phosphorus), and 1% is in a pool that can exchange with extracellular calcium

Factors Affecting Ca^{2+}Absorption
- Caffeine decreases
- Fibres decrease (soluble)
- Magnesium and zinc decrease
- Clacitrial and PTH increase
- Pregnancy and lactation increase

Ca^{2+} Deficiency and Toxicity

Deficiency
- Profoundly affects bone and muscle
    - Bone
        - Inadequate mineralization in bone
            - Rickets in children (commonly associated with vitamin D deficiency)
            - Osteoporosis in adults, sometimes osteomalacia
    - Muscle
        - Tetany
            - A condition characterized by intermittent muscle contractions that fail to relax
- Evidence for association with hypertension
- Evidence for association with colon cancer (current research supports association in high risk populations)

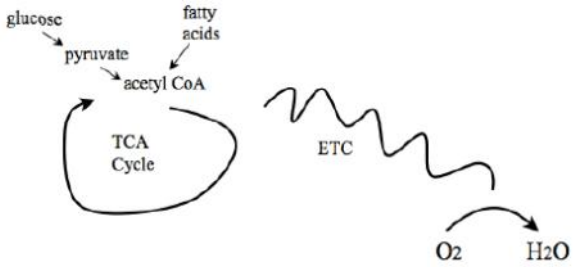
Toxicity
- Constipation, bloating, and/or gas
- Hypercalcemia
    - Kidney stones

Phosphorus
- Widely distributed in food, so deficiency and toxicity are very rare, however very important in physiology (2nd most abundant mineral in body)
- Found in both animal products (as phosphorus) and in grains (as phytic acid)
    - Most phosphorus absorbed as an inorganic ion
        - Passive diffusion (primary method)
        - Saturable, carrier- mediated, active transport (NaPi co-transporter)
    - Primarily in duodenum and jejunum
    - 50-70% phosphorus in foods absorbed
    - Absorption inhibited by magnesium, aluminum and calcium
- Transported in blood bot 70% organic phosphate (e.g. in phospholipids) and about 30% HPO_4^{2-}
- Predominantly found in bone, but also central metabolism (ATP, DNA, RNA, cAMP, etc)
- Plays a key role in protein phosphorylation (common PTM in proteins)
- Impritant in cell membranes, bones metabolism and PTM of proteins

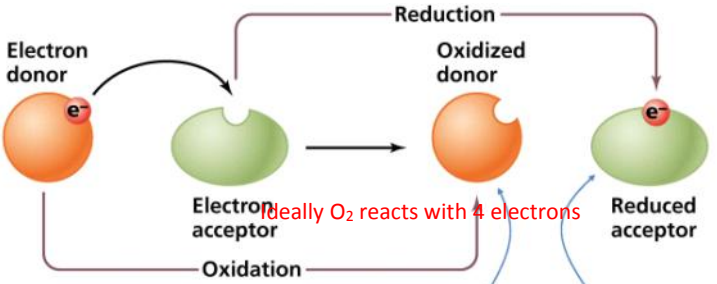
Fluoride
- Fluoride present in body in trace amounts; not essential
- Community water fluoridated (with about 1 ppm or 1 mg/L) for the past 60 years due to the inverse relationship between fluoride intake and dental caries
- Absorption in stomach by passive diffusion (nearly 100% efficiency)
- Transported in the body as ionic fluoride or bound to plasma
- Major function related to effects on mineralization of teeth and bones
    - Increases resistance of enamel acid demineralization by forming fluoropatite (protective layer)
- Deficiency
    - Increased incidence of tooth decay
- Toxicity
    - Fluorosis (mottling of teeth)
    - Tolerable upper limit in adults is 10mg/day

Micronutrients Part 2- Involved in Oxidant Defense

Reduction- Oxidation Reactions

- Reduction-oxidation reaction is a redox reaction
    - Transfer of electrons between two substrates
    - Biochemical reactions are essentially electron transfers
- Large number of our nutrients have redox functions
- Principle electron carriers--> NADH and $FADH_2$
- Why is this important
    - Creation of ATP
- 

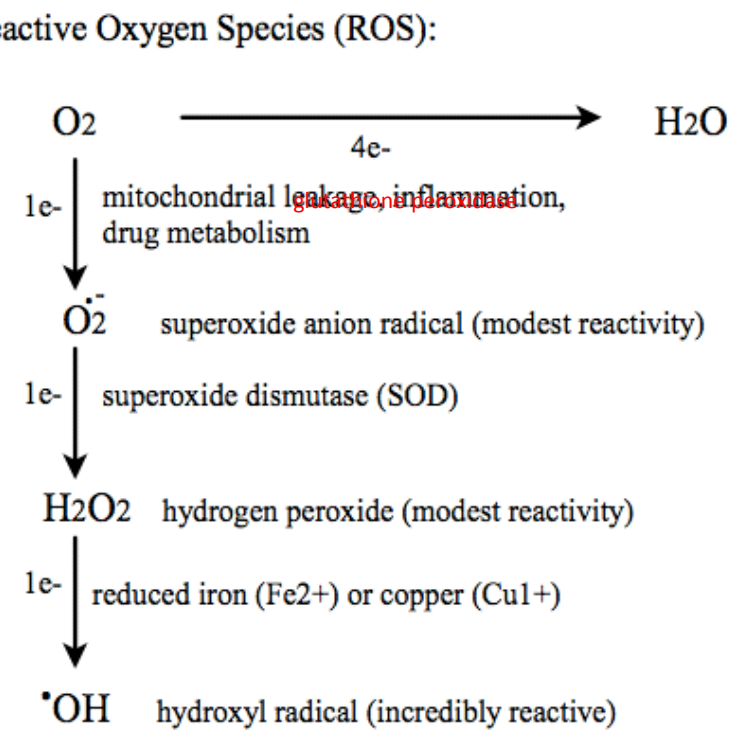
Redox Reactions

- 

O_2 reacts with one electron

Reactive Oxygen Species (ROS)

O_2^- reacts with one electron

- 

H_2O_2 reacts with one electron

- ROS produced as a by-product of the ETC where electron flow fails (~1%)
- Occurs in a O_2- rich environment, so oxygen can bind electrons
- Mutations is SOD = Lou Gehrig's
- H_2O_2 converted to H_2O by catalase, glutathione peroxidase (selenium dependent), etc

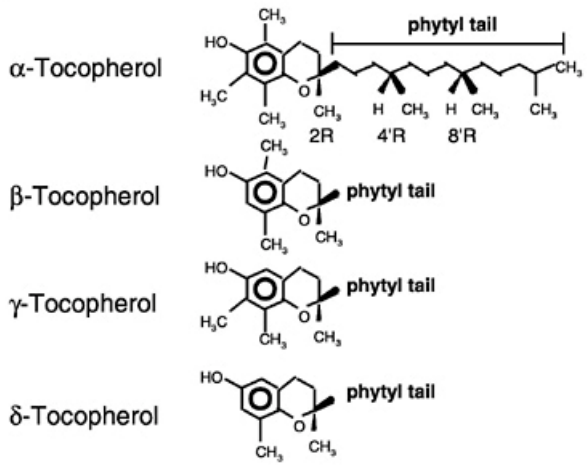
- Making OH is not a good thing, the entire cell can die with the production of one OH
- Attack macromolecules
    - DNA
    - Lipids
    - Leads to cancer and cell death

Vitamin E

- Encompasses 8 compounds (viatmers)
    - 4 tocopherols
        - Have saturated side chains with 16 carbons
    - 4 tocotrienols
        - Have unsaturated side chains with 16 carbons
- Vitamers in both classes (alpha, beta, gamma, sigma?)
- Only alpha- tocopherol has a biologic acitivty
    - 7 of the 8 do not have important biological activity
- Can not interconvert vitamers
- All are found naturally in goods
- Tocopherol derived from Greek word:
    - Tokos = "childbirth" and Phero = " to bear or bring forth"
    - Based on work showing that rats couldn't reproduce when vitamin E was absent from the diet

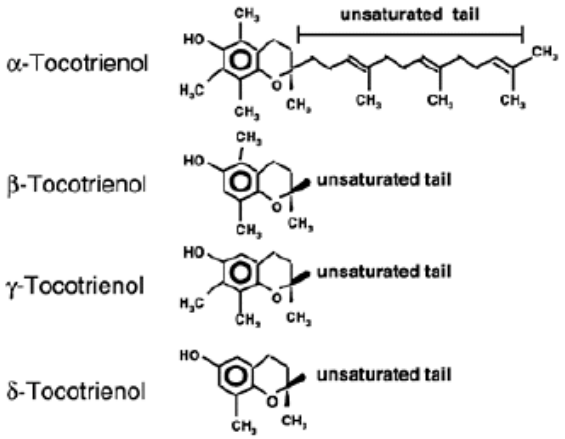
4 Tocopherols

- Saturated side chain (phytyl tail)
- Nomenclature used to describe number and position of ring CH_3 groups
- Alpha = most methylated
- The hydroxyl group (circled) is an antioxidat site which is very necessary
- R and S configuration determined by first 3 CH_3 groups on side chain
- Natural alpha- tocopherol is RRR
    - Fits in tocopherol transfer protein (TTP)
-



4 Tocotrienols

- Unsaturated side chain (phytyl tail)
- Rules for nomenclature the same
- Originally thought that tocotrienols don't contribute towards vitamin E requirements (absorbed, but not converted to alpha- tocopherol or recognized by TTP
- New evidence questions ths

- 

Vitamin E Intake

- Food sources
    - Nuts, seeds, vegetable oils, leafy greens
    - Susceptible to food preparation and storage (e.g. roasting nuts decreases vitamin E content)
    - Mostly from plants because stored in fat animals
- Dietary recommendations based on alpha-tocopherol only
    - Estimated based on crude tests examining RBC hemolysis (releases haemoglobin) in presence of dilute H_2O_2 (>20% = deficiency)
- RDAs for vitamin E in "mg" or "IU"
    - Adults (15 mg = 22.4 IU) of RRR-a-ttocopherol per day
    - Increased in pregnant woman
- UL = 1000 mg/day (causes increased bleeding)
    - But gastrointestinal problems seen at lower intakes
- Deficiency is very rare (would have to be less than 5 ug/mL plasma)
    - Pre- mature infants
    - Individuals with fat malabsorption disorders
    - Genetic defects in lipoproteins or TTP

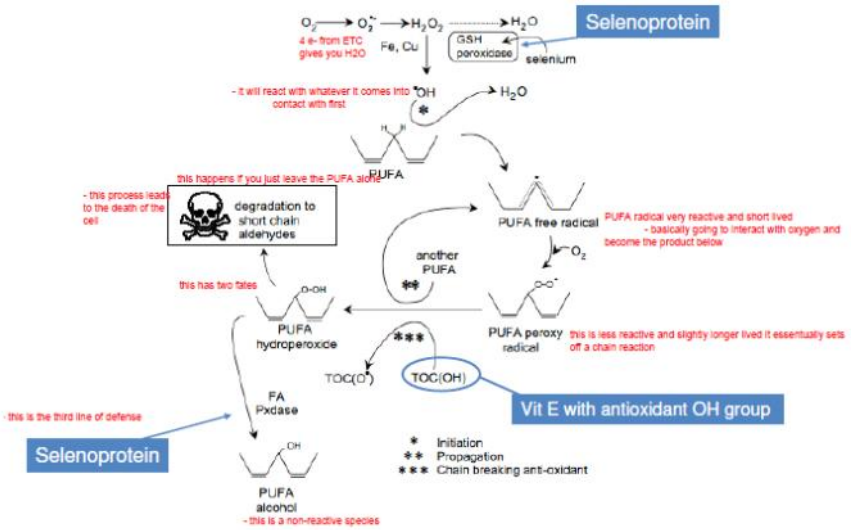
Digestion, Absorption and Metabolism

- Absorbed in jejunum
- Bile salts necessary
- NPC1L1 transporter thought to be involved in uptake
- Packaged in chylomicrons
- CR arrive at the liver
- Liver makes TTP, which assists alpha-tocopherol into VLDL
    - Other isomers help a bit with anti-oxidant activity, but are quickly degraded and lost
- In cells, vitamin E located in membranes
- >90% vitamin E in body in adipose tissues as fat droplets

Vitamin E Function - Antioxidant

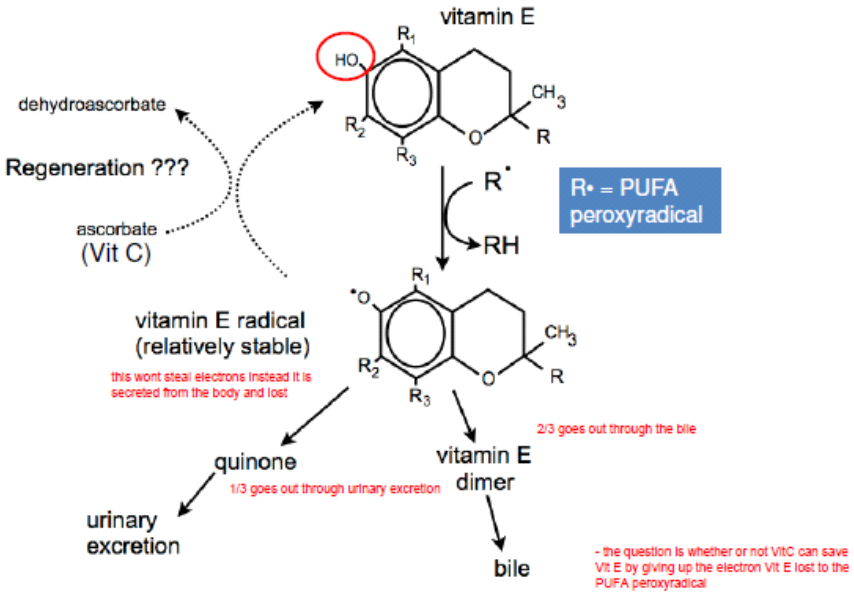
- This process happens in every cell with mitochondria
- Lines of defense include
    - GSH peroxidase
    - Vitamin E
    - FA peroxidase

-



Vitamin E Function - Further Metabolism

-



  - Regeneration of active vitamin E by vitamin C happens in virto, but conflicting results in vito

<u>Selenium</u>

- Associated with protein (plant proteins)
  - Plants incorporate selenium instead of sulfur into protein
- Selenium content in food determined by soil content
  - Relationship with cancer is pretty low
    - In region where there are low levels of selnium there is a rise in disease
    - Selenium is important as seen in the last diagram slife, you can't put up a fight against PUFA which leads to cell death and possibly a relation to cancer
- Absorbed in small intestine by AA transport systems
- SelenoAA travel freely in blood
- Selenocysteine is the ultimate goal of the body (aslo made from selenomethionine)
- 30 selnoproteins in the body
- Deficiency
  - China and Africa (low in Se in soil)
  - Keshan disease
- Toxicity

- Selenosis (rare)

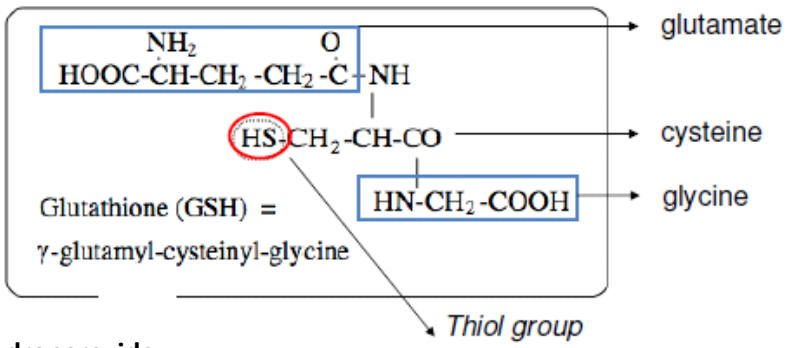
Selenoproteins

- Glutathione peroxidase is the first line of defence against lipid peroxidation
- Fatty acid peroxidase is the third line of defence against lipid peroxidation
  - Both selenoproteins, and contain selenocysteine
- Both use of GSH (gluthione) as substrate
- Primary role -->protect organism from oxidative damage
- Require glutathione (GSH), which acts as the reducing agent
- Structure is crucial, as glutamate and custeine linked via gamma carbon
  - This gamma peptide bond is resistant to cellular proteases
- Gluathione is the major intracellular reducing agent (about 10mM in liver, Tripeptide)

This bond is resistant to proteases

The SH group is the electron donor, it gives the electron to the radicals to stop having radicals

Even though it got rid of an electron , it is really stable because it is between glutamate and glycine, it can be brought back to normal by some process the cell does



PUFA Hydroperoxide

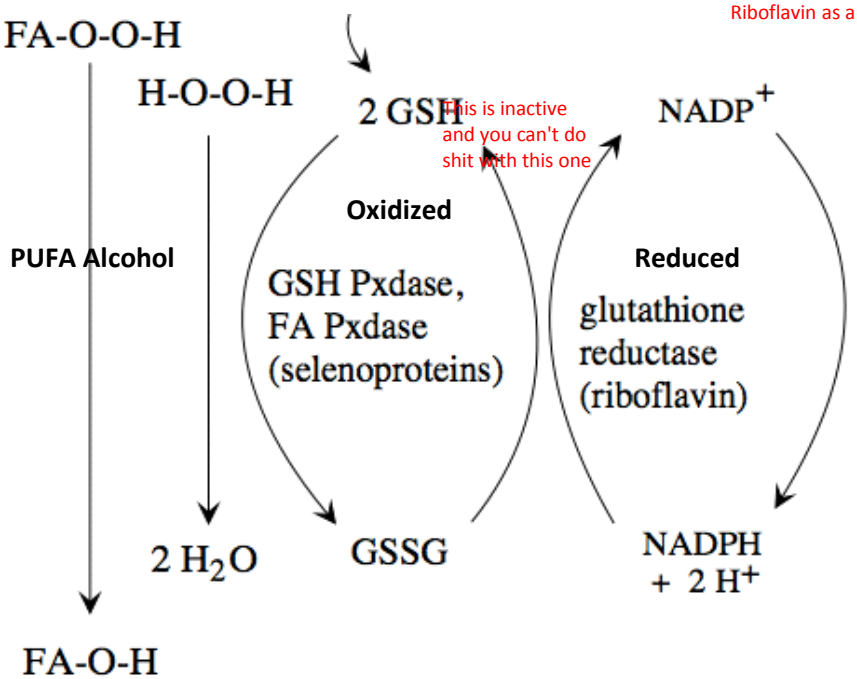
Reduced **Oxidized**

Glutathione (GSH)

Pentase-phosphate Pathway to become NADPH again

- Thiol group of cysteine donates 1 e^-
- GSH becomes reactive with another GSH to form GSSG
- Healthy cell has >90% GSH and <10% GSSG
  - Lower GSH indicative of oxidative stress
  - This means that it is probably in the process of dying
- Oral GSH supplements not very effective (poorly absorbed)

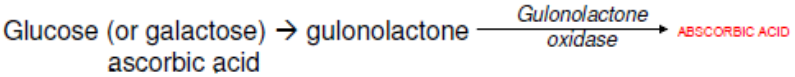
Riboflavin as a cofactor

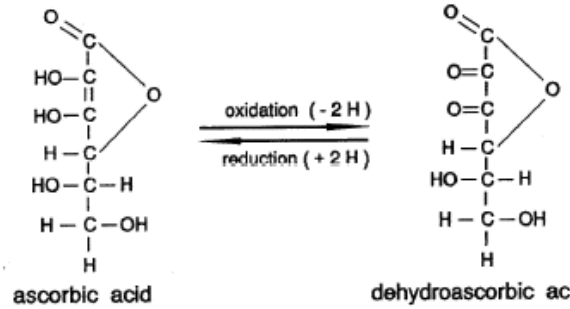


-

Vitamin C

- Vitamin C (ascorbic acid)
  - At physiological pH = ascorbate
- Exists in both D and L-isomers
  - L-isomers is biologically active in humans
- Most mammals can synthesize vitamin C from glucose, except
  - Humans, primates, fruit bats, guinea pigs, and some birds
  - This is because we lack gulonolactone oxidase
  - Uronic Acid Pathway



- Primary source is fruits and vegetables
- Sensitive to heat, light, oxidation and alkaline solutions
- High dietary Fe or Cu promotes oxidative destruction of dietary vitamin C
- Doesn't require digestion prior to absorption
- Transport via sodium-dependent vitamin C (SVCT) 1 and 2 in the small intestine (feedback mechanism exists)
  - 70-90% dietary vitamin C is absorbed
    - The more vitamin C consumed the lower the amount absorbed
- Found in circulation primarily in free form
  - It chills in the body as is
- In foods, mostly ascorbic acid, but small amounts of oxidized form (dehydroascrobic acid) exists
- Tissue concentration of vitamin C plasma
- Involved in a number of processes
  - Collagen synthesis
  - Tyrosine synthesis
  - Neurotransmitter synthesis
- Vitamin C acts primarily as a reducing agent in these processes
- The two biologically active forms are:
  - 

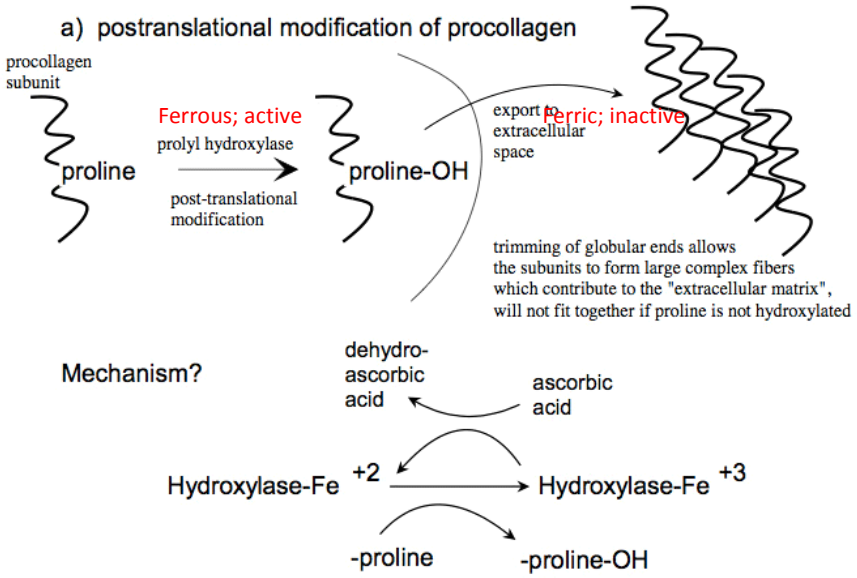
ascorbic acid (reduced)

dehydroascorbic acid

30% AA in procollagen = Pro (1-3) of these are hydroxylated

Pro-OH is what allows the collagen molecules to stick together

Hydroxylation Reactions

- 

-

Oxidant Defense

- If there is vitamin C around, there is some evidence that reactions lipid perocidation happens to a lesser extent
- And this is evidenced by lower levels of lipid peroxidation products being measured in the urine
- In the neutrophils, where we have a lot of oxygen radicals, there are higher levels of vitamin C
- During vitamin C deficiency, there is some increase in GSSG (oxidized dimer form that is not longer active) and a decrease in GSH (the active form of glutathione). It is not clear how this occurs

Requirements and Deficiency

- RDA: the goal is to maximize tissue concentrations and minimize urinary excretion
    - Men 90mg/dat
        - RDA increases with pregnancy and lactation
    - Extra 35mg/day for smokers
        - Smoking increases oxidative stress and vitamin C use
    - UL >2g/day leads to GIT problems (diarrhea)
- Signs of deficiency
    - Scurvy (plasma vitamin C levels <0.2 md/dL)
        - If you consume 10 mg vitamin C per day, scurvy would occur in one month
    - Hemorrhages (skins, follicles, gums)
    - Hair loss, loose teeth
    - Swollen joints, poor wound healing
        - These are due to problems producing hydroxyproline

<u>Summary of Biochemical Functions</u>

- Vitamin E
    - Scavenges PUFA peroxy radicals within the membrane environment
- Selenium
    - Essential for the proper functioning of selenoproteins
        - Glutathione peroxidase (prevents hydroxyl radicals and converts H_2O_2 into water)
        - FA peroxidase (prevents PUFA peroxy radicals and converts into PUFA alcohols)
    - Vitamin C

        - Major blood reducing agent, but it's role in oxidant defence remains unclear
    - Sulfur AA
        - Required for GSH synthesis
            - Thiol group in the cysteine structure
    - Niacin
        - Required to make NADPH (needed to regenerat GSH by glutathione reductase)
    - Riboflavin also required by glutathione reducatse as a coenzyme
    - Finally Cu and Zn involved in converting superoxide into H_2O_2
- Together, all of these vitamins and minerals necessary for antioxidant systems

<u>Micronutrients Part 3- Micronutrients that act as Enzyme Cofactors</u>

<u>Niacin</u>

- Also known as vitamin B3
    - Discovered through the condition pellagra in humans and similar condition, called black tongue, in dogs
        - The "anti-black tounge" factor
    - High incidence in areas where corn is the main dietary staple (because niacin is bound to complex carbohydrates and poorly absorbed)
- Generic term for
    - Nicotinic acid and nicotinamide
- Dietary sources? Most fish, meats, breads, and cereals, coffee and tea
    - In coffee, trigonelline is converted to niacin by heat (coffee bean roasting)
- In animal foods, niacin occurs predominantly as
    - Nicotinamide
    - Nictinamide adenine dinucleotide (NAD)
    - Nicotinamine adenine dinucleotide phosphate (NADP)
- In plant foodsm niacing predominantly as nicotinic acid
- Niacin can also be produced in the liver from the AA tryptophan (but only about 1/60th of Trp converted into niacin)

Digestion and Absorption

- Digestion of NAD and NADP required for absorption of niacin
    - Hydrolyzed by glycohydrolase to release free nicotinamide
- Nicotinic acid and nicotinamide absorbed a bit in stomach, but mostly in small intestine by facilitated diffusion
- In plasma , niacin found primarily as nicotinamide
    - 1/3 of the nicotinamide bound to plasma proteins
- Both forms can diffuse across cell membranes in most tissues except kidney and RBC (carrier-mediated)
    - Nicotinamide --> primary precursor for NAD (all tissues)
    - Nicotinic acid --> used for NAD in only the liver
- Once NAD or NADP produced, this essentially traps niacin within the cell, typically found as
    - NAD^+ (oxidized form)
        - Primary function is transfer electrons to the ETC
    - NADPH (reduced form)
        - Primary function is reducing agent in biochemical pathways

NAP Production

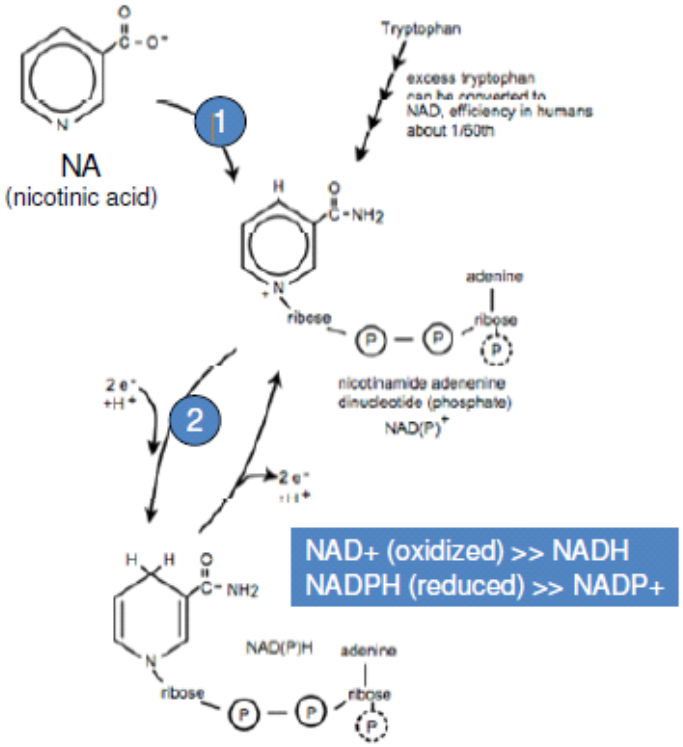
- From either NA or Trp
- Two steps:
    1. Convert the acid to an amide

2. Build into a dinucleotide sturtcure

- NAD^+ reduced to NADH in
  - Glycolysis, Kreb's cycle and beta oxidation
  - Catabolic
- $NADP^+$ reduced to NADPH in hexose monophosphate shunt and used for
  - Fatty acid synthesis, DNA synthesis, glutathione regeneration, etc
  - Anabolic
- Accept 2 electrons
- Over 200 enzymes require NAD and NADP as coenzymes

Dinucleotide because it has two bases, two phosphates groups and two sugars (ribose)this is in the oxidized form

-



Requirements and Deficiency

- Corn/maize
  - Contains significant amounts of vitamin B3 but it's not readily absorbed
  - Also deficient in Trp
  - Use of lime, from limestone can help release vitamin B3 from corn
    - Used by Native Americans in food practices
- Deficiency leads to pellagra
  - 4 Ds:
    - Dermatitis
    - Dementia
    - Diarrhea
    - Death
    - These are all reversible
- RDA recommendations include niacin produced from Trp
  - Therefore, niacin equivalent (NE)
  - NE = mg preformed niacin + mg Trp/60
  - 14/16 mg per day NE women/men

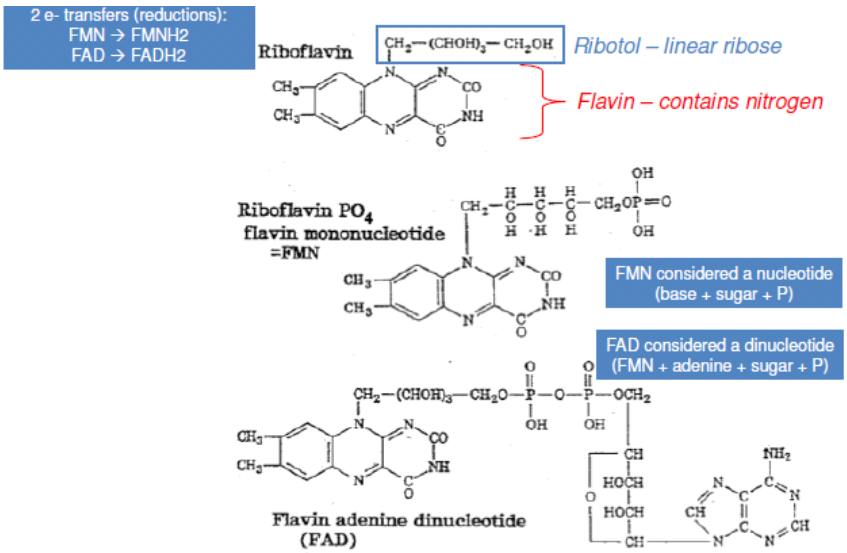
<u>Riboflavin</u>

- Also known as vitamin B2
  - Riboflavin stems from "ribo" (ribose-like side chain) + "flavus" (yellow color)
- Rich is foods with animal origin
  - Milk, milk products, meat, etc (source of free riboflavin)

    - Other foods (found as either flavin mononucleotide (FMN) of flavin adenine dinucleotide(FAD))
    - Sensitive to sunlight
- Ribolfavin bound to proteins must be freed proper to absorption which is done by HC; in the stomach
- FAD/FMN converted to free riboflavin for absorption
- Free riboflavin absorbed by a saturable, energy-dependent, sodium-dependent carrier (riboflavin transporter 2, RFT2)
- Alcohol inhibits riboflavin digestion and absorption
- Riboflavin, FAD, FMN transported in blood by proteins like albumin
- Body stores (liver, kidney, and heart) sufficient for 2-6 weeks
- FMN and FAD produced in cells
    - FMN is the major form (60-95%)
    - Production positively regulated by T3 hormone (activates flavokinase)
    - FMN/FAD involved in redox reactions

Metabolism

-

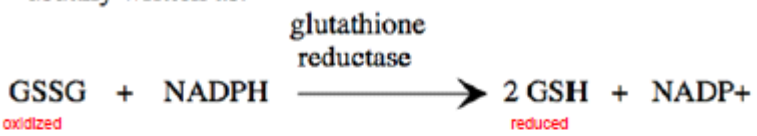


    - Riboflavin turns into riboflavin PO_4 by means of flavokinase (driven by Mg^{2+} or Mn^{2+} and the usage of ATP)
    - Riboflavin PO_4 turns into Flavin Adenine Dinucleotide by means of FAD synthetrase (Driven by Mg^{2+} or Mn^{2+}) and the usage of ATP

Riboflavin - Redox

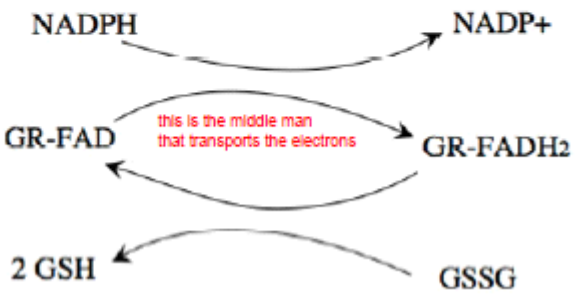
- FMN and FAD function similarly to NAD(P)
- Difference is that they are tightly bound to active site of enzymes and responsible for passing the electrons
- Accepts 2 electrons from NADPH
- Typical function for riboflavin in many biochemical pathways
    - Beta oxidation
    - Kreb`s cycle
    - Delivery of electrons to ETC
- So reforming glutathione requires both niacin (NAD) and riboflavin (FAD)

- usually written as:



what actually happens:

-



  - GR-FAD --> glutathione reductase + FAD (coenzyme)

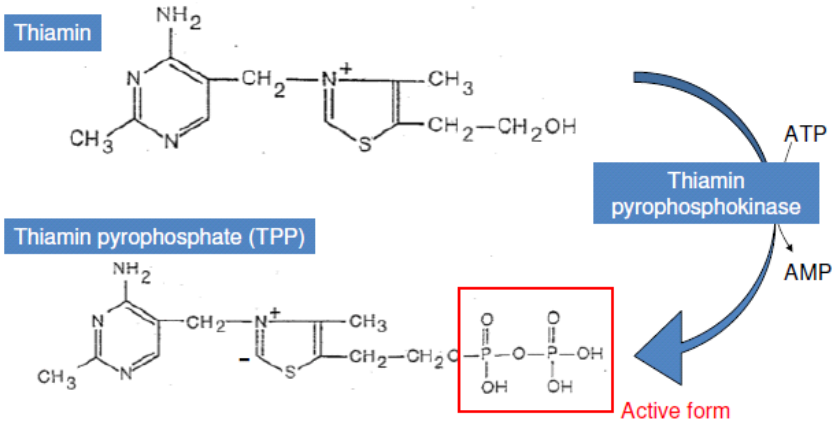
Requirements and Deficiency

- Requirements
  - 1.3 mg/day in men and 1.1. mg/day in women
- Deficiency
  - Deficiency signs appear ~ 3-4 months
  - Deficiency called Ariboflavinosis
  - Deficiency related common when dietary intake is insufficient as it is continuously excreted in the urine
  - Now clear riboflavin deficiency has been characterized
  - Cracked and red lips, inflammation of the lining of the mouth and the tongue, mouth ulcers, cracks at the corners of the mouth aka angular cheilitis
- At risk populations
  - People with thyroid disease
  - Chronic consumption of alcohol
- No UL for riboflavin

Thiamin

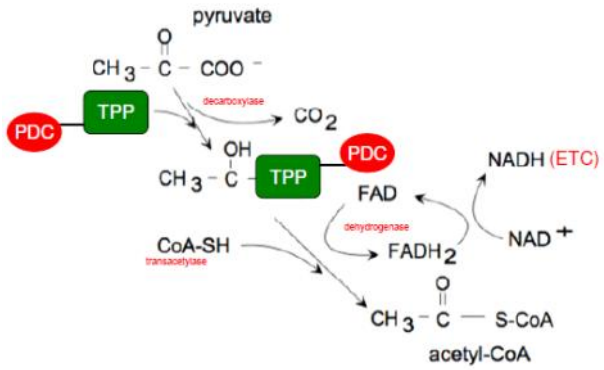
- Also known as vitamin B1
- Need for thiamin first discovered in the 1800s when birds fed a diet of cooked polished rice developed neurological problems (beriberi)
- In a number of foods: meats; whole fortified or enriched grain products
  - Typically found in foods containing riboflavin and niacin
  - Destroyed by heat and alkaline environment
    - So cooking foods in hot water will lead to loss of thiamin
- In plants, thiamin exists in free form
- In animals, thiamin exists in phosphorylated form (thiamin pyrophosphate; TTP)
  - Phosphate group must be removed before absorption
- Absorption occurs primarily in jejunum and ileum
  - Typically controlled by thiamin transporters
  - Alcohol can reduce thiamin uptake by decreasing production of transporters
- In plasma, thiamin found in it's free form, bound to albumin

TTP Production

- 

- Thiamin is an important cofactor for pyruvate dehydrogenase (pyruvate --> acetyl CoA) and alpha-ketoglutarate dehydrogenase (Kreb's cycle)

Thiamin in Energy Metabolism

- TTP important in two oxidative decarboxylation steps
    1. Pyruvate dehydrogenase complex
    2. Alpha-ketoglutarate dehydrogenase complex
- Require riboflavin (FAD), iacin (NAD), and pantothenic acid as cofactors
- Critical in the movement of sugars and amino acids into energy metabolism pathways
- Thiamin is also required by the transketolase pathways in the hexose monophosphate shunt, thereby playing a role in NADPH production and synthesis of ribose
- Thiamin triphosphate (TTP) is present in nervous tissue and play's a poorly understood role in nervous transmission
- 3 enzymes in complex
    - Pyruvate decarboxylase (PDC)
    - Transacetylase
    - Dehydrogenase
- Micronutrients
    1. Pyruvate decarboxylase --> Thiamin
    2. Transacetylase --> Pantothenic acid
    3. Dehydrogenase --> Riboflavin (FAD) and Niacin (NAD)
- 

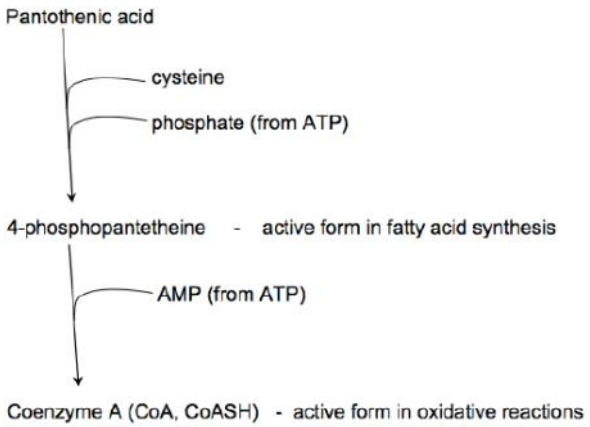
Requirements and Deficiency

- RDA for men is 1.2 mg/day and for women 1.1 mg/day
    - Differences due to differences in body size and energy needs
- Deficiency
    - Interferes with critical pyruvate and alpha-ketoglutarate dehydrogenase complexes
        - Prevents ATP and acetyl-CoA synthesis
        - Accumulation of pyruvate, lactate and alpha-ketoglutarate in blood

- Dry beriberi
    - Predominantly in adults due to chronic low thiamin intake
    - Muscle weakness, peripheral neuropathy affecting distal limbs
- Wet beriberi
    - More common in children and young adults
    - Affects the cardiovascular system
- Acute beriberi
    - Occurs in infants
    - Anorexia, vomiting, lactic acidosis
- At risk? Increases with age
    - In USA, thiamin deficiency commonly associated with alcoholism
    - Populations depending on polished rice as a major source of food
- No UL established for thiamin

Pantothenic Acid

- Essentially not discovered until 1954
- Once called vitamin B5
- Derived from the greek word pantos, which means "everyywhere"
    - Present everywhere (virtually all plants and animal foods); therefore deficiency next to impossible
- Occurs in foods in both free and bound forms
    - ~85% pantothenic acid in food occurs as part of coenzyme A (CoA)
- Absorbed in the jejunum by passive diffusion
- Found free in blood (within RBC)
- Uptake into organs dependent on sodium-dependent multivitamin transporter (SMVT)
- Essential in energy metabolism (formation of acetyl CoA) allowing Kreb's cycle to take place
- Active group is SH (from cysteine)



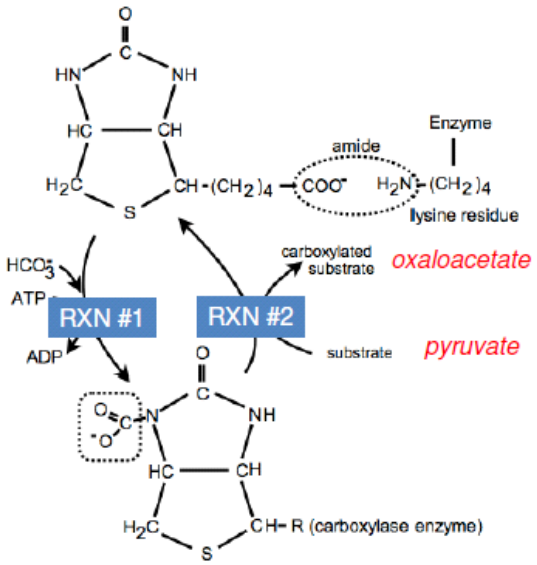
    - Beta-oxidation of fatty acids
    - Acetyl CoA, succinyl CoA, propionyl CoA

Biotin

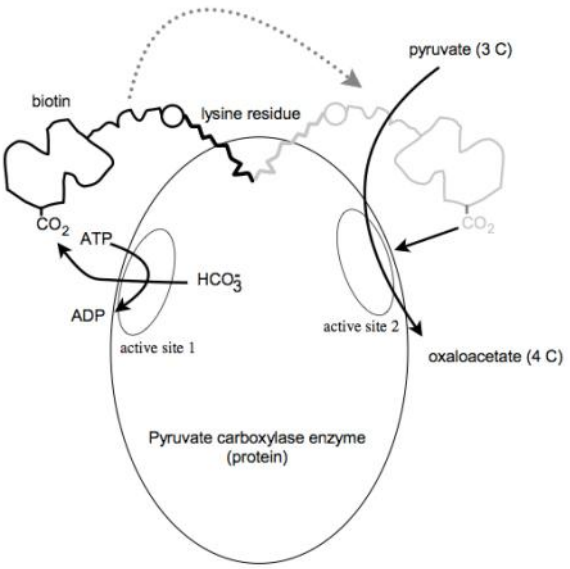
- Discovered in 1931 during experiments examining the cause of egg white injury
    - Eating raw eggs led to hair loss, dermititis, etc (discovery of avidin, a biotin-binding protein)
    - Originally termed "Vitamin H"
- Made by intestinal bacteria (although not sufficient to meet the biotin needs of humans)
- Widely found in foods bound to proteins
- Must be cleaved from proteins prior to absorption (proteolysis by pepsin)
- Free biotin is absorbed to near completion

    - Alcohol inhibits biotin absorption
- Circulates in blood in the free form (~80%), with a bit bound to albumin
- No RDA or deficiency, it's so highly prevalent

Biotin-Mediated Carboxylation

- Two rings (with N and S) and a side chain with a Carboxyl group
- The carboxyl group interacts with the NH_3 group of a lysine residue in enzyme (e.g. pyruvate carboxylase)
- Two-step reaction
    1. Carboxylating the N in the biotin ring structure
    2. Transferring the CO_2 to another molecule
- 3 key reactions involving biotin
    1. Pyruvate carboxylation (production of oxaloacetate)
    2. Malonyl CoA formation
    3. Conversion of propionate into glucose
- 

Biotin Function (A Closer Look)

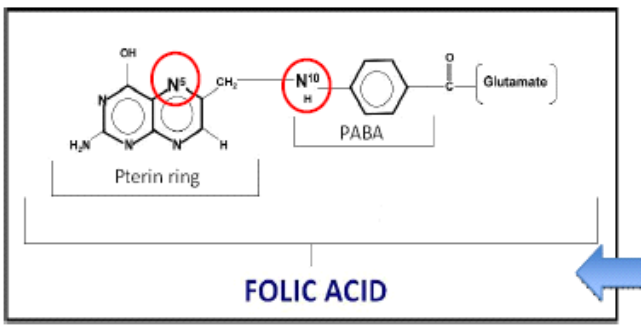
- Pyruvate carboxylase requires a biotin cofactor to be active
- The biotin is anchored to a lysine side chain, allowing it to reach into the carboxylation reactive site (active site #1), which carboxylates the biotin ring
- The cofactor can then swing into the active site #2, brining the carboxyl group into close proximity with substrate
- 

Folate

- Folate and B12 were discovered during the search to cure megaloblastic anemia, a problem in the late 1800s
- Folate and folic acid are not interchangeable
    - Folic acid--> oxidized form of the vitamin found in fortified foods and supplements (100% biovailable)
    - Folate --> reduced form of the vitamin found naturally in fods (50% bioavailable)
- Folate composed of 3 parts, all have to be present for vitamin activity
    - Pterin ring; PBA (para-aminobenzoic acid); glutamic acid
    - Humans can synthesize all 3 components, but do not have the enzyme to conjugate them (i.e. join them together)
- In the body, metabolically active folate has multiple glutamic acid residues attached (i.e. polyglutamate)
- Digestion polyglutamates must be broken down to the simplest form (monoglutamate) before absorption takes place
    - Enzymes are sensitive to alcohol and enzyme inhibitors naturally present in certain foods like legumes and lentils
    - Folic acid already a monoglutamate structure
- Absorbed by proton-couple folate transporter (PCFT) in small intestine
- In plasma, mostly found as folate and 3-methyl THF
    - Mostly bound to proteins, such as albumin

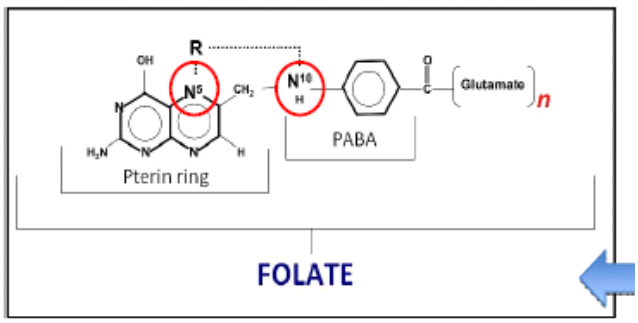
Folate vs. Folic Acid

-



    - Only 1 glutamate reside
    - Pteroylmonoglutamate

-



    - Multiple glutamate residues
    - Pteroylpolyglutamate

Cobalamin

- Also known as vitamin B12 was the last vitamin to be discovered
- Generic term for a group of compounds called coronoids (because of corrin nucleus)
    - Contains cobalt (very rare)
    - Most complex Vitamin structure
- Bacteria produce vitamin B12
- Dietary sources primarily from animals (stored in tissues)

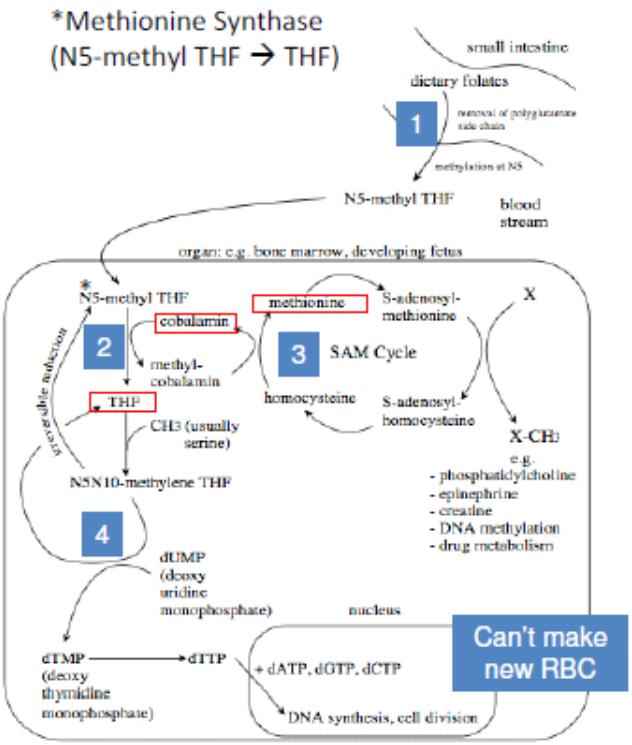
    - Vegans at risk of deficiency
- Very focused use in metabolism
- Vitamin B12 deficient
    - Functional deficiency for folate (the two work together)
    - Neurological problems
- RDA 2.5 ug/day

Vit B12 Absorption

- In stomach, a specific binding protein secreted from gastric lining (intrinsic factor, IF)
- Vit B12- IF complex goes to a receptor in the ileum
- Complex broken down in the enterocyte, and Vit B12 absorbed while IF released back into intestinal lumen
- Vitamin B12 stored in liver and undergoes enterohepatic circulation
- Highly stored in the liver, so deficiencies may take years to surface
- Two types of vitamin B12 deficiency
    1. Not enough in your diet - in affluent vegans (resolved with megadoses of vitamin B12)
    2. Improper absorption due to defects in IF
    - Deficiencies showin in blood in the form of megaloblastic anemia

<u>Single Carbon Metabolism</u>

- Major dietary CH_3 donor groups
    - Choline, Serine, Methionine
- Cofactors
    - Folate (N5- methyl THF or N5,N10-methylene THF (tetrahydrofolate)
    - Vitamin B12 ("prosthetic" group)
    - S-adenosyl methionine (SAM)
- Folate deficiency --> impaired DNA synthesis and DNA repair; uracil misincorporation, megaloblastic anemia
-



The Folate Trap

- Only reaction that can metabolize N5-methul THF is methionine synthase (vitamin B12 dependent)

- B12 deficient, N5- methyl THF trapped
- Large doses of dietary folate can overcome B12 deficiency
- 5X RDA saturates ability to form N5-methyl THF, so you get more free folate going to the liver where you produce THF (bypass the trap)
- Still have problems with the SAM cycle

Folate Deficiency

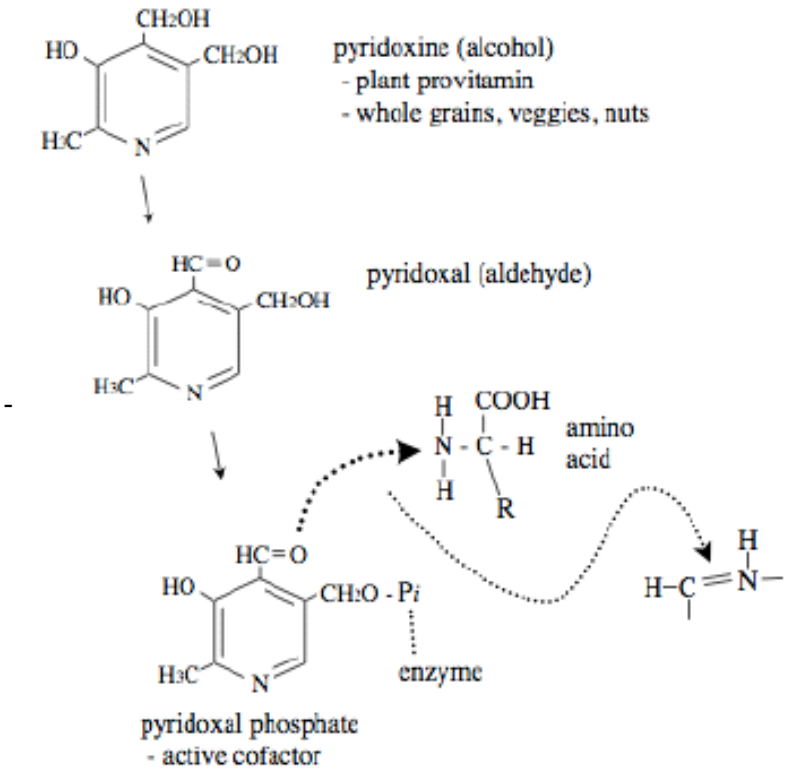
- Folate is critically important in dividing cells, which supports the production of specialized cells that forms the neural tube
    - This takes place during early pregnancy, so folate deficiency leads to sever birth defects (neural tube defects; NTD)
    - In the embryo, the neural tube is the CNS, which becomes the brain and spinal cord
        - NTD is an opening of the spinal cord or brain
        - Closed vs. open NTD (open more common): brain or spinal cord exposed

Vitamin B6

- Initially found while trying to understand dermatitis in rates
- Exists as 6 vitamers which are interchangeable and comparably active
    - Pyridoxine, pyridoxal, pyrdioxamine
    - All 3 can be phosphorylated
- All isomers are found in foods which means deficiencies in humans is rare
- High protein intake demands more Vitamin B6
- Must be dephosphorylated prior to absorption
    - Passive diffusion in the jejunum
- PLP is the main form in blood, bound to albumin
- Major storage site is in the muscle

Functions

- Transamination
- 1st step in porphyrin synthesis
    - Uses glycine and succinyl CoA from Kreb's (hemoglobin, myoglobin, cytochromes in ETC)
- Synthesis of neuroactive amines (epi & norepinephrine serotonin, histamine, GABA)



Micronutrient Deficiencies and Anemia

- Stem cells in bone marrow become differentiated in presence of erythropoietin (EPO)
- Normal process is that differentiation causes cells to shrink, nucleus removed and able to carry O_2
- Process is very sensitive to micronutrient deficiencies
    - This can lead to anemia
- There are three types of anemia
    - Megaloblastic anemia
        - This is a result of a deficiency in folate, and B12
        - RBCs are too big, but too few and they become stuffed with hemoglobin
        - In this anemia the deficiency impairs the activity of the enzyme thymidylate synthase which is requires for DNA synthesis
        - As a result the RBCs can't be made in the initial stages
    - Hemolytic Anemia
        - Result of a deficiency in vitamin E, selenium and cysteine
        - There are not enough RBCs due to premature destruction of the cells
        - High oxygen in RBCs leads to the production of a lot of radicals and when absent there is a lot of lipid preoxidation of the membrane which ends up in the cells dying
    - Micorcytic Anemia
        - Result of a deficiency in vitamin B6
        - RBCs are small and pale due to a deficiency of B6 affecting the synthesis of porphyrin
        - Without adequate porphyrin, you don't have normal synthesis of hemoglobin and the cells are very pale
        - There are also very small because hemoglobin is what gives them bulk

Micronutrients Part 4 - Essential Trace Minerals

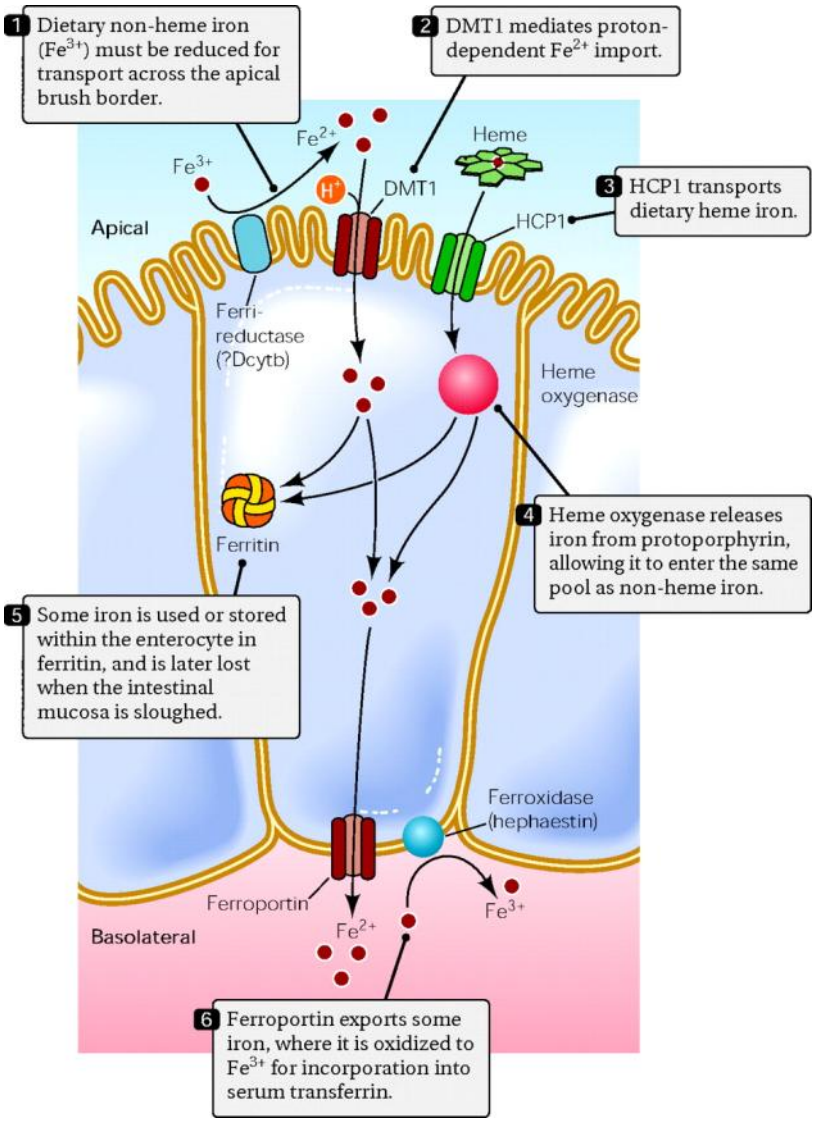
Iron

- Widely distrubuted in foods at low amounts
    - Liver, meat products, plant sources (leafy green vegetables, fruits, nuts)
- Within food, iron is found in one of two forms
    - Heme (within the porphyrin ring of hemoglobin and myoglobin) - Animals
    - Non-heme - plants
- Only two states of iron are stable in the aqueous environment of the body and in food Fe^{3+} (ferric) and Fe^{2+} (ferrous)
- Functions of iron in body
    - Oxygen transport
        - Hemoglobin
        - Myoglobin
    - Redox - active component of ETC (iron sulfur centers and cytochrome heme proteins)
    - Iron metalloenzymes
- RDA: 8mg per day in men, 18mg per day in women and 27 mg per day in pregnancy
- UL: 45 mg per day (hemosiderosis)

Different Forms, Different Bioavailability

- 3 forms of iron in our diet
- Between 10-18% ingested iron absorbed but it depends on the person's iron status
- Non-heme iron
    - HCl and proteases cleave non-heme iron from food components in stomach releasing mostly ferric (Fe^{3+}) iron
    - Stomach acid most converst Fe^{3+} into Fe^{2+} (ferrous)
    - Fe^{3+} reduced by a reductase into Fe^{2+}
    - Fe^{2+} absorbed via a divalent metal transporter 1 (DMT 1)
- Heme iron
    - Released from hemoglobin/ myoglobin by HCl proteases in stomach
    - Heme (porphyrin ring) taken up in a small intestine by heme carrier protein 1 (HCP1)
    - Inside cell, heme porphyrin ring hydrolyzed by heme oxygenase into Fe^{2+} and protoporphyrin

-

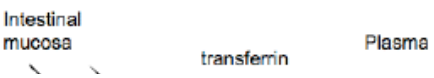


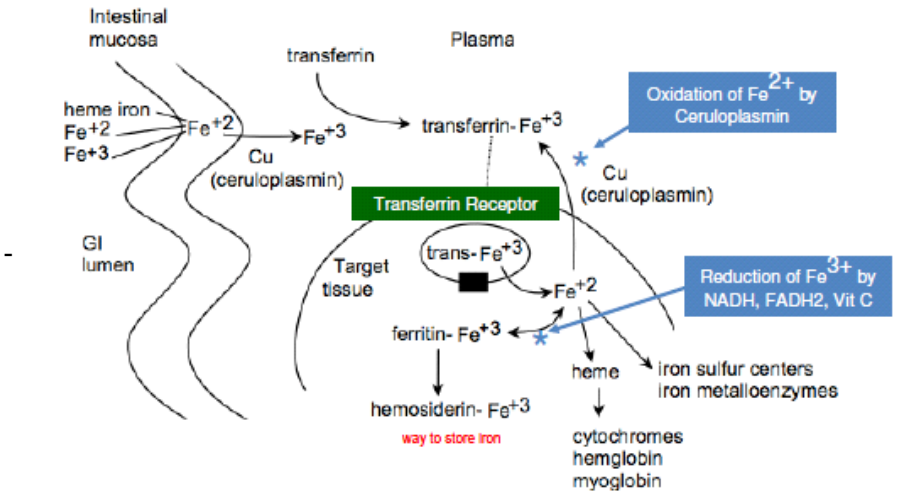
Factors Influencing Absorption

- Chelators --> small organic compounds that form a complex with a metal ion
    - This can affect iron absorption
    - If iron-chelate is soluble then absorption is enhanced
    - If Iron-chelate is soluble then absorption is inhibited
- Enhancers include sugars, vitamin C, digestion production from meet, poultry and fish, soluble fibres
    - Vitamin C acts as a reducing agent and chelator with ferric iron
- Inhibitors include polyphenols (in tea and coffee), oxalic acid (spinach), insoluble fibres
    - Coffer just after a meal reduces iron absorption by about 50%
    - Oxalic acid and chelates with iron (zinc and copper too)

Iron Distribution and Metabolism

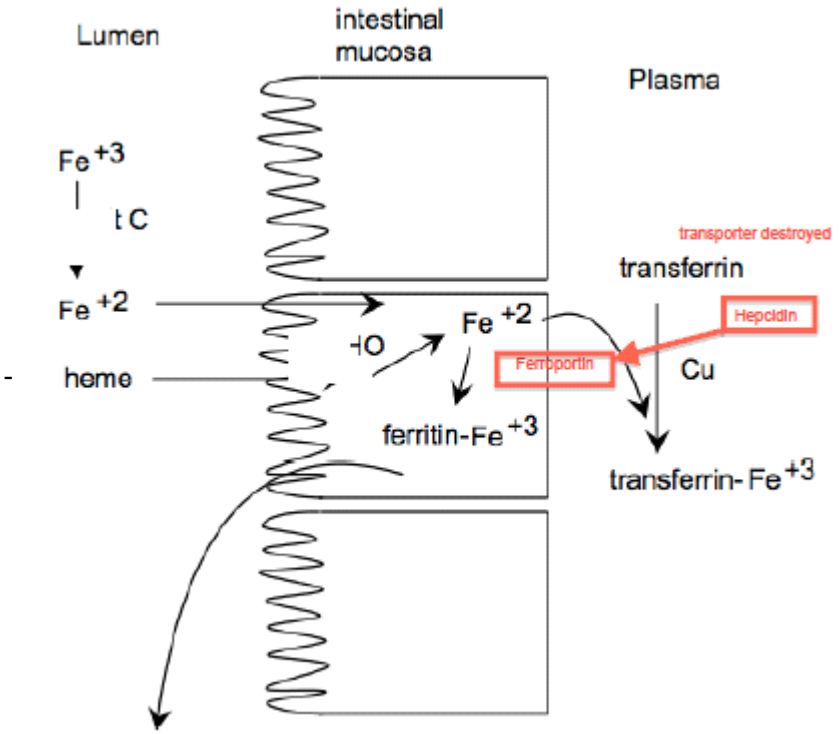
- Iron is transported around the body in the ferric form bound to carrier proteins
    - The primary carrier protein is transferrin
- This is important because when iron is left unbound it has left reox activity which can lead to the production of harmful free radicals that interact with H_2O_2
    - This also helps avoid bacterial proliferation (bacteria can't access the iron)





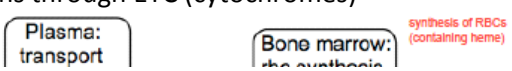
Regulation of Iron Levels in the Body

- Iron status
  - High = >40%
  - Low = <15%
- Liver senses diferric transferrin levels
- High levels prompt production and secretion of hepcidin
- Transferrin has 2 binding ites
  - Normal plasma iron levels (33% transferrin bound with Fe^{3+})
- Intestinal enterocytes have a half-life of 3 days and are then lost in the lumen
  - Iron is lost in the feces when iron isn't able to escape so in about three days or so when the cell dies the iron gets excreted in the feces

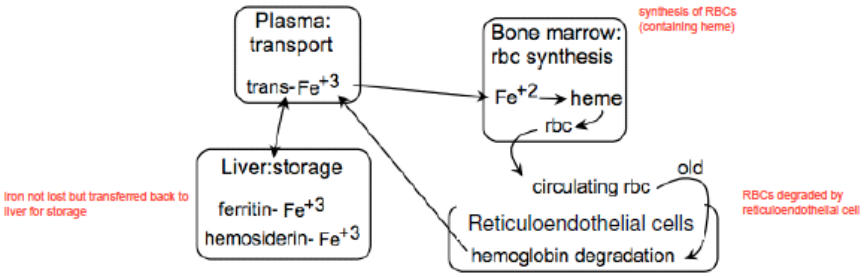


Important Tissues in RBC Hematopoiesis

- Haematopoiesis is the formation of blood cellular components (iron needed for RBCs)
- Iron is essential due to its presence in heme, which enables oxygen transport to tissues (haemoglobin), oxygen storage (myoglobin) and transport of electrons through ETC (cytochromes)

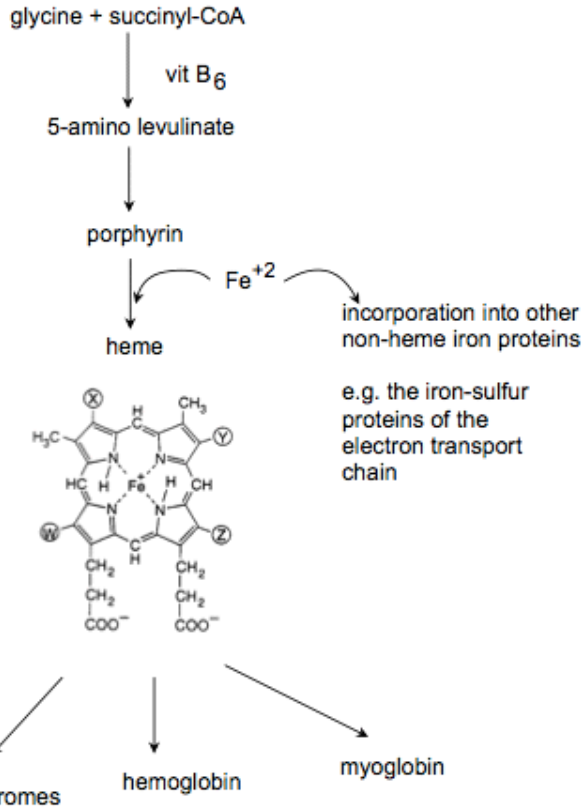


-



Iron and Heme

- Porphyrin synthesis starts with glycine and succinyl-CoA and requires vitamin B6 (which is why a B6 deficiency can lead to anemia)



  - Other spots where heme is important
    - Catalase (antioxidant role)
    - Converts H_2O_2 into H_2O
  - Thyroperoxidase
    - Addition of iodides are added to thyroglobulin

Iron Deficiency and Toxicity

- Most often due to inadequate iron intake in four groups
  1. Infants and young children
  2. Adolescents in their early growth spurts
  3. Females during childbearing years
  4. Pregnant women
- Short term consequences of Iron deficiency anemia
  - Lower test scores on mental development
  - Lower test scores on motor development
  - Variable improvement in test scores after treatment with iron
    - In many other words, some functional changes may be permanent
  - Other symptoms include
    - Fatigue, pallor weakness, hair loss, irritability, brittle or grooved nails, impaired immune function

        - Hemochrmatosis (increased iron absorption) due to deficient hepcidin
    - Hemachromatosis (increased iron absorption) due to deficient hepcidin
- These can be treated with iron supplements or eating iron rich foods

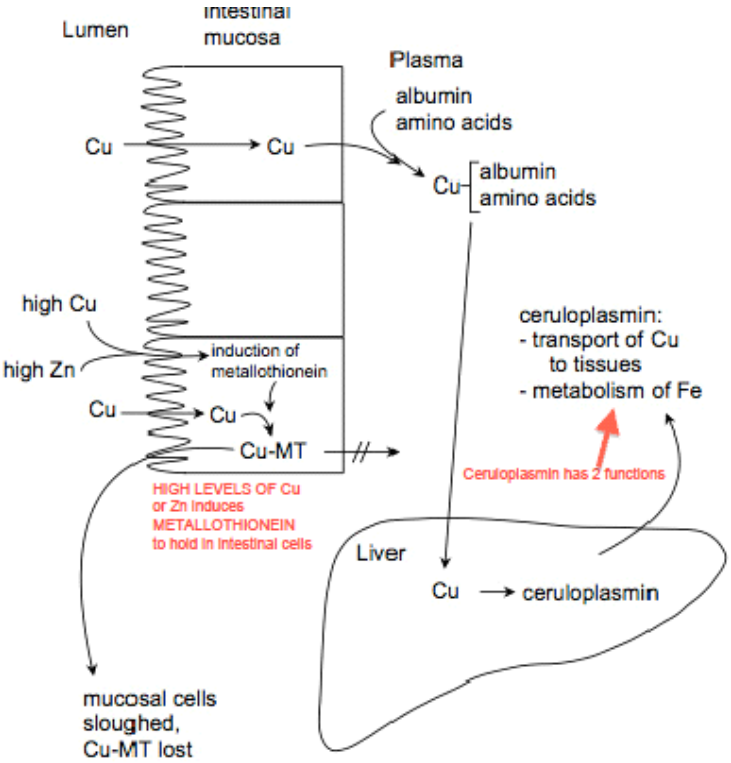
Copper

- Content varies widely and reflects origin, and conditions of production handling and preparation
- Sources include oysters and shellfish, whole grains, beans, buts, potatoes, organ means and dark leafy greens
- Functions as a component of many important enzymes, including:
    1. Fe metabolism - Ceruloplasmin
    2. Energy production - cytochrome c oxidase
    3. Antioxidant - superoxide dimutase
    4. Norepinephrine production (begins with Tyr) - dopamine monoxygenase
    5. Melanin formation - copper dependent enzyme tyrosinase produces pigments
- Acts as an enzyme cofactor, often as an intermediate in electron transfer (redox reactions)
- RDA: 0.9 mg
- UL: 10 mg

Copper Absorption and Distribution

- Cu^{2+} bound to amino acids
    - HCl and pepsin free Cu^{2+}
- Mostly in small intestine, a bit in stomach
- Copper carrier protein (copper transporter 1:Ctr1) DMT1
- ~50% ingested Cu^{2+} absorbed
- Enhancer chelators
    - Amino acids
    - Organic acids (citric)
    - Vitamin C
- Inhibitor Chelators
    - Antacids
    - Phytic and (P form plants)
    - Zinc

-



Copper Deficiency - Menke's Disease

- General symptoms include, anaemia, hypopigmentation of skin, bone abnormalities, etc
- Menke's Diseases (aka Menke's Kinky Hair Syndrome)
    - X-linked genetic disease
    - Low serum copper and ceruloplasmin
    - Inborn error of metabolism in which body cannot absorb enough Cu^{2+}

Copper Excess - Wilson's Disease

- Symptoms includes drooling, slurred speech, problems swallowing, problems walking, cognitive impairment
- Causes are:
    - A buildup of copper in the body
    - Excess copper is normally excreted by the body, however this cannot be done by people with Wilson's disease due to a mutation on the ATP7B gene
    - When the copper storage capacity of the liver is exceeded, copper is released into the bloodstream and travels to other organs including the brain, kidney and eyes
- Treatments are a low copper diet, chelation therapy and zinc therapy

Zinc

- Found in all organs, tissues and body fluids
- Sources
    - Oysters contain more zinc per serving than any other food but red meat and poultry provide the majority of zinc in the North American diet
    - Other good food sources include beans, nuts, crab lobster, whole drains, fortified breakfast cereals and dairy products
    - Found complexed with nucleic acid and AA in peptide/proteins
- Functions
    1. Zn containing metalloenzymes - more than 200 known at present and involved in all pathways in metabolism

        - Provides structural integrity --> stabilizes tertiary structure
        - Participates in reaction at catalytic site (accepts or donates e^-)
    2. Oxygen radical metabolism - component of Cu/Zn SOD
    3. Zinc finger - DNA binding
- Examples include
    - Carboxypeptidase (protein digestion)
    - Superoxide dismutase (antioxidant(
    - Polyglutamate hydrolase (folate digestion)
    - Nucleic acid synthesis (DNA and RNA polymerase)

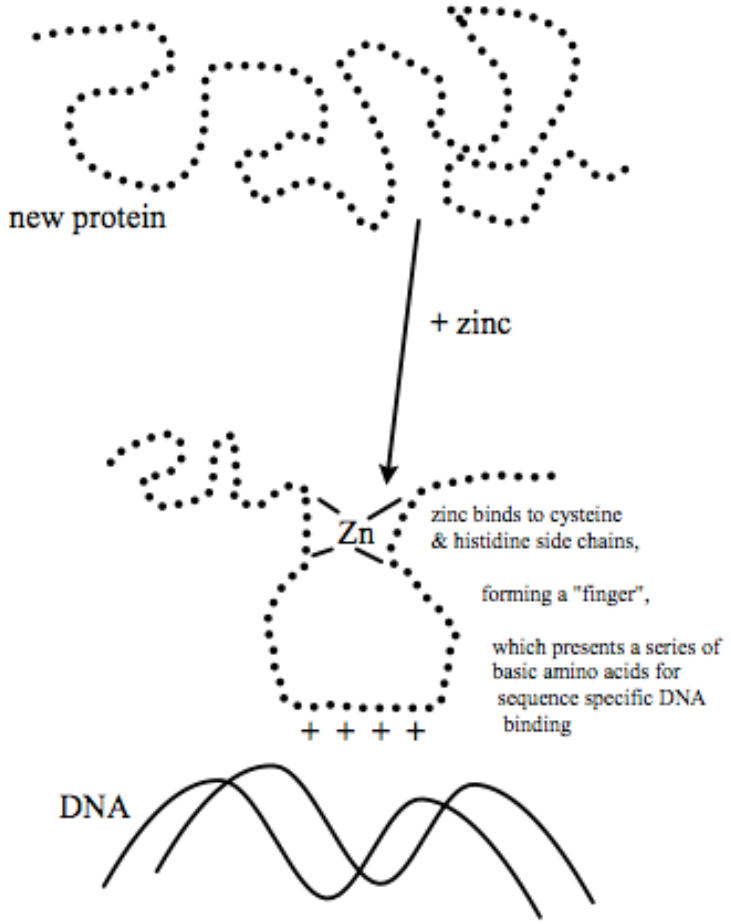
Digestion and Absorption

- Absorption
    - Like iron, zinc must be hydrolyzed from amino acids before absorption
        - Acid environment of stomach and digestive enzymes in smal intestine
    - Of ingested zinc --> 20-30% absorbed (higher than iron, lower than copper)
        - Two mechanisms: carrier-mediated (ZIP4; primary mechanism) and diffusion
    - Absorption increases when zinc status is low (promotes ZIP4 degradation)
    - Enhancer chelators
        - Organic acids
        - Prostaglandins
    - Inhibitor chelators
        - Antacids (zinc absorption efficient in acidic environment)
        - Phytic acid, oxalic acid
        - Folic acid, Iron, Calcium
        - Metallothionein induced by high dietary copper in intestinal cells is also induced by hight dietary zinc, so a high zinc diet can induce a copper deficiency
            - Holding on to zinc when it is produced lowers copper's availability by holding on to it as well
- In enterocytes, zinc can be
    - Used functionally
    - Stored (by binding with metallothionein)
    - Secreted into circulation and transported bound to albumin (first destination is the liver, and then other tissues)

Zinc Fingers and DNA Binding

- Major role in regulating gene expression
    - If you don’t have zinc fingers you wont have interactions
- Zinc fingers
    - Used to describe the shape of transcription factor proteins when bound to zinc
    - Interactions between zinc and side chains of histidine and cysteine
    - Enables basic amino acid to interact with DNA

-



Zinc Deficiency and Toxicity

- RDA is 8 mg per day in females and 11mg per day in males
    - Accounts for zinc losses (primarily via GIT)
    - Unabsorbed zinc, sloughed off enterocytes containing zinc, zinc-containing enzymes used for digestion
- Deficiency
    - Common in humans (especially elderl, vegetarians, children
    - Poor absorption induced by phytic acid in grains
    - In children, growth retardation (inadequate cell division), poor wound healing, delayed sexual maturation (deficient testosterone synthesis), impaired taste (insufficient taste proteins)
        - You can't replicate DNA properly which leads to insufficient proteins
- Toxicity
    - UL: 40 mg per day
    - Leads to copper deficiency due to induction of metallothionein
    - Neurological problems, numbness, metallic taste, nausea, etc.