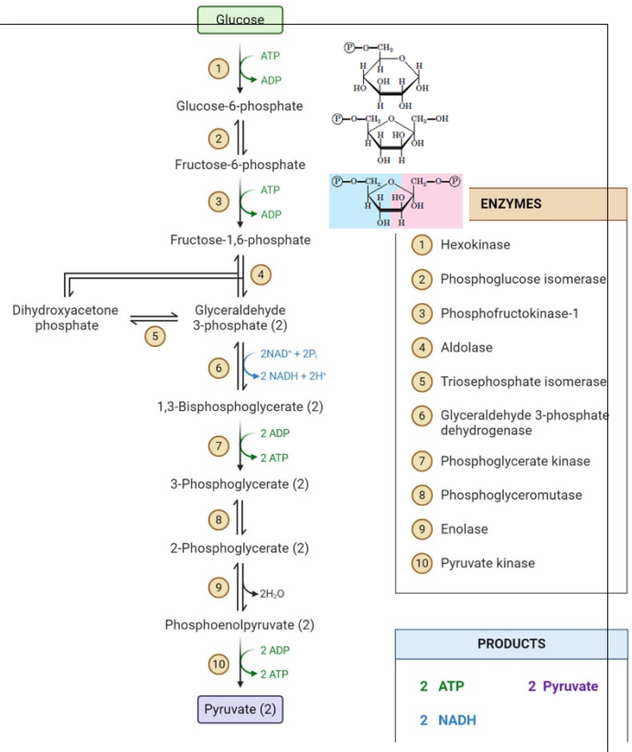
Glycolysis



- **Why:** Oxidation Glucose → Pyruvate for energy/intermediates via substrate level phosph + high phosphate group transfer potential
- **Where:** Cytosol, glucose transported via Na+ independent diffusion(GLUT) , Na+ cotransporter (Depends on NA/K pump)
- **Regulation:** ATP and Citrate inhibit PFK1, AMP and F2,6BP activate PFK1, G6P inhibits Hexokinase, F1,6BP activates pyruvate kinase
- **Hormonal Regulation:** Insulin increased glycolysis, glucagon→cAMP → Inhibits pyruvate kinase, decrease it
- Steps 1, 3, 10 are irreversible and key regulatory, highly exergonic
- Steps 1, 3 require energy input, 7 and 10 each create 2 ATP
- 2 ATP in (1 from glycogen), 4 ATP out, preparatory and payoff phases
- Glucose can become: Pyruvate, Ribose-5-phosphate, glycogen, structural sugar
- Pyruvate can become: Acetyl-CoA, Lactate, Ethanol, Alanine, Fatty acids
- Warburg Effect-Tumors

Pentose Phosphate Pathway

- Alternative fate of G6P, occurs in cytosol, shunted off glycolysis
- Oxidative phase: produces NADPH needed for fatty acid and cholesterol synthesis, and ribose-5-P for nucleotides
  - ⇒ Gluc-6P to Ribulose-5P forms NADPH using Gluc-6P dehydrogenase enzyme
  - ⇒ Ribose-5-P for DNA/RNA, NADPH for Fatty acid biosynthesis and antioxidant ability

  Non-Oxidative phase: converts pentose sugars to glucose-6-P vis 2 glyceraldehyde-6-P or 1 fructose-6-P
- NADPH inhibits Gluc6P dehydrogenase
- No ATP consumed or produced

Cori Cycle

- Lactate from fermentation into liver, converted to glucose via gluconeogenesis, released in blood
- Cori cycle Pyruvate + NADH ⇔ Lactate + NAD+

Gluconeogenesis

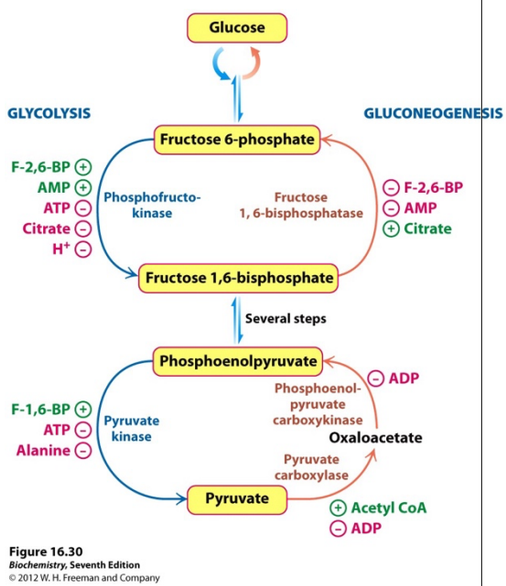


Figure 16.30
Biochemistry, Seventh Edition

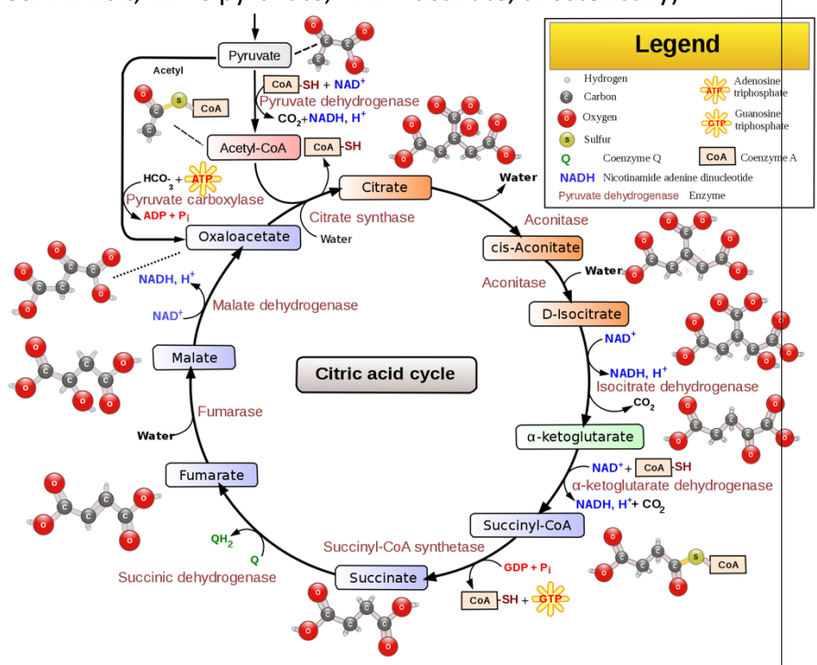
- Synthesis of Glucose from non-carbohydrate precursors, when glycogen used up
- Happens in the cytosol except pyruvate → Malate→ Oxaloacetate in mitochondria, in liver and renal cortex cells
- Starting ingredients can be Lactate, Pyruvate, Glucogenic Amino acids, Glycerol, and TCA intermediates through oxaloacetate
- Steps 1, 3 and 9 of glycolysis are irreversible, negative ΔG, bypass necessary
- Bypass mechanisms:
  1. Pyruvate→Oxaloacetate→PEP via Pyruvate carboxylase, then PEP carboxykinase, the first requiring ATP and CO2 and the later needing GTP and releasing CO2
  2. F1,6BP needs H2O and produces P, uses F1,6-Bisphosphatase
  3. GTP to Glucose also needs H2O, yields P, uses G6-Phosphatase
- Regulatory is the opposite of glycolysis, Le Chateaus principle:
  - Insulin decreases glucose levels (less gluconeogenesis), FED state
  - Glucagon/Epinephrin Increase glucose levels (more gluconeogenesis), Fasted state
- Hypoglycemic individuals lack G6-Phosphatase, cannot circulate glucose from liver

Krebs/TCA

- Anaplerotic(makes intermediates) and amphibolic (both catabolism and anabolism) process
    - Citrate → Fatty acids/Sterols, a-keto-gluterate → Glutamate → Arganine or Purines, Succinyl-CoA → Hemes, OAA → PEP/Glucose or Asparatate/Pyrimadines
- Complete oxidation of glucose to H2O and CO2 in mitochondrial matrix
- Pyruvate enters matrix through pyruvate-H+ symport
- Reduce 4 NADH and 1 FADH2 + GTP per pyruvate
- Pyruvate Dehydrogenase enzyme (PDH) has E1,2,3 uses CoASH, FAD, TPP and NAD+ as cofactors, removes CO2 from Pyruvate, adds S+CoA group, very irreversible reaction

Regulation

- Pyruvate dehydrogenase is regulated by:
    - Feedback inhibition (NADH, Acetyl-CoA inhibit, while pyruvate, NAD+ activate, allosterically)
    - Covalent modification: phosphatase activates in response to Ca+ or Mg+, kinase deactivates in responses to ATP, NADH, Acetyl-CoA, all allosterically
- No hormonal control directly, allosterically controlled:
    - NADH inhibits all NADH creating steps (0,3,4),
    - High energy like succinyl-CoA (1,4) and ATP (0, 3) inhibit
    - ADP activates (0, 3), Ca+ activates (0,3,4) in muscles



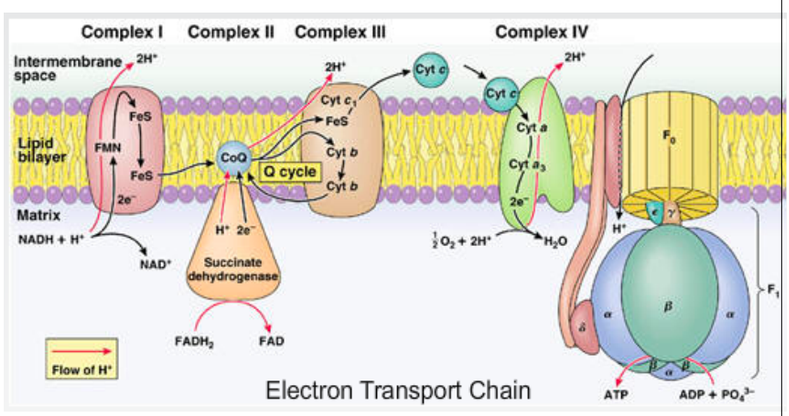
Oxidative Phosphorylation

Shuttles

- Malate-Aspartate Shuttle: Cardiac Liver cells, OAA → Malate-transport→OAA → asertatate-transport, OAA+NADH⇔Malate
- Glycerol-3P Shuttle: Brain, skeletal musc, Glycerol-3P → FADH2 + Dehydroxyacet-P + NADH → Glycer-3P, Between CII and CIII

Electron Transport Chain

- Oxidation of NADH and carriers yields energy to pump hydrogen to intermembrane space, reducing O2
- Iron-Sulfur: Fe2+ ⇔ Fe3+
- Flavins: Accept-donate 1 or 2 electron
- Cytochromes: Iron Heme Fe2+ ⇔ Fe3+
- ATP-Synthase, F1 in matrix rotating, F0 is the tube Y rotates, a and b stationary, binding change model, loose/tight/open, 120 degree steps



- Oxidative phosphorylation of 3 ATP per 360 degree rotation, 3 binding sites
- Regulated by ATPvsADP concentration
- NADH = 10 H+ = 2.5 ATP, FADH2 = 6H 32ATP total

Glycogen

- Glycogen granules-branched homopolymers in liver, provide glucose for brain and muscles
- Formed around glycogenin protein, a(1→4) and a(1-6) at branching points
- High glucose induces glycogenesis, low induces glycogenolysis, if glycogen full excess glucose becomes fat
- Stored in muscles and liver

Glycogenolysis

1. **Glycogen phosphorylase** cuts a(1-4) on reducing ends yielding glucose-1-phosphate
2. **Hydrolysis** by the debranching enzyme removes the a(1-6) glycosidic bond so 1 can continue
3. **Isomerization** to glucose-6-phosphate by phosphoglucomutase enzyme
4. **Dephosphorylation** to glucose so the sugar can enter the blood and be delivered to other tissues

- Two fates of Glucose-6-P

Glycogenesis

1. **Isomerization** of G6P to G1P by phosphoglucomutase
2. **Activation** by the transfer of UMP (Uracil monophosphate) to G1P, forming UDP-Glucose through pyrophosphylase
3. **Glycogen Synthesis** by glycogen synthase enzyme, cleaving the UDP and using the energy to from a(1-4) with glycogen chain

Regulation

- Reciprocal control through phosphorylation:
  - Glycogen phosphorylase (activated by cAMP-low glucose, inhibited by G6P)
  - Glycogen synthase (Stimulated by G6P, inhibited by cAMP)
- Hormonal: Insulin activates glycogen synthase, glucagon and epinephrin inhibit it
  - Insulin from pancreas to liver in response to increased glucose, stimulates glycogen synthase, inhibits breakdown
  - Glucagon from pancreas to liver stimulates glycogen breakdown, long term effects by activating gluconeogenesis
  - Epinephrine from adrenal gland to liver and muscles stimulates rapid glucose mobilization, as well as glycolysis ect.

Lipids

Digestion

1. Triacylglycerides → Digested by lingual, and gastric lipases into free fatty acids, or di/mono glycerides
2. Pancreatic lipase cuts so only monoglycerides or FFA , Bile in small intestine emulsifies forming micelles
3. Bile salt coated micelles enter small intestine mucosa and converted into TAGs

Transport

1. TAGs incorporated with cholesterol, phospholipids, proteins forming Chylomicrons, able to transport in blood
2. Lipoproteins activate lipase using Apo-C2, FFAs broken down in mitochondria of adipose tissue or muscles
3. Remnants of chylomicrons transported to liver to form VLDL, end of exogenous pathway, start of endogenous
4. From liver, VLDL move through blood to capillaries, activating lipase proteins with Apo-C2, releasing FFA muscles, adipose
5. IDL formed from VLDL, can be further cleaved releasing FFA and forming LDL, or can reuptake by liver via Apo-E
6. LDL can be phagocyted (Clathrin) by cells needing cholesterol(58%), or reuptake by liver via Apo-B100
7. HDL from the liver collect free (bad) cholesterol from tissues, forming IDL or serving as lipoprotein storage

Lipid related Proteins

- LCAT: helps maturation of HDL to LDL
- ACAT: Esterification of cholesterol and Acetyl-CoA for limiting the solubility of cholesterol in membranes and Chylomicrons
- CETP: Exchanges cholesteryl esters and TAGs from VLDL/LDL ⇔ HDL

Lipid Biosynthesis

- In Cytoplasm of Liver: Glucose → Acetyl-CoA → Malonyl-CoA → Fatty Acid → Lipids or TAGs
- 8 Acetyl-CoA + 7 ATP + 14 NADPH → Palmitic acid (16) + 7 ADP + 7 P + 14 NADP+ + 6 H2O + 8 CoA
- **Regulation targets** Acetyl-CoA carboxylase (Rate limiting), Citrate activates it, and Palmityl-CoA inhibits it Insulin activates Acetyl-CoA-Carboxylase, glucagon inactivates it through phosphorylation

1. **Transport** of Acetyl-CoA from mitochondrial matrix to cytoplasm via formation **Citrate transporter**
   - OAA + Acetyl-CoA → Citrate transporter → OAA → Malate transported to reform OAA and Pyruvate
   - Acetyl-CoA transported to cytoplasm, Pyruvate transported in, Costs 1 ATP, regenerates NADP to NADPH
2. **Activation:** by Carboxylation of Acetyl-CoA → Malonyl-CoA by Acetyl-CoA Carboxylase by adding COO- group
   - Uses Biotin as cofactor, committed step, rate limiting step
3. **Chain elongation** by **Fatty Acid Synthase (FAS**), Adds 2 Carbon per cycle until Palmitic acid (16C) is reached:
   - Once FAS binds Malonyl-ACP by Malonyl-CoA-ACP transacetylase, and Butyryl-KS FAS is charged
     1. **Condensation**: of butyryl-KS and Malonyl-ACP forming keto-acyl-ACP by K-A-Synthase removing CO2
     2. **Reduction:** to form hydroxyacyl-ACP, uses one NADPH (removes double bond to O)
     3. **Dehydration:** to form a C-C double bond, removes water, trans-enoyl-ACP formed, H2O leaves
     4. **Reduction** of the C-C double bond to form butyryl-ACP, uses another NADPH
     5. **Transfer:** The butyryl group is transferred from the ACP site to the Cystine site, making way for another round
   - NADPH come from PPP and **Malic enzyme** (Malate → pyruvate + NADPH + CO2)
   - 3 of these Palmitic acids join with glycerol to form TAG

- Fatty acids can be used to for phospholipids in high growth, or TAG for energy storage
- TAG and Phospholipids both require conversion of fatty acid to **Phosphatic Acid** first
  - Phosphatidic acid is attached to a polar head to form Glycerophosphates, and backbone elongation if TAG
- Glycerol-3P is an important precursor for TAG and phospholipids, and can be made from Pyruvate via Glyceroneogenesis, needed for TAG formation
- Sphingolipids are synthesized from Palmityl-CoA and serine, through desaturation followed by head attachment (Cerebroside)
- Cholesterol is needed for membranes, and a precursor to bile, hormones and is made in the liver
  - Cholesterol is synthesized from Mevalonate, which comes from Acetyl-CoA → HMG-CoA followed by reductase
  - Mevalonate is a 6C, made into cholesterol a 27C in peroxisomes, ER and cytosol, costing 18 ATP and 14 NADPH

Beta Oxidation

- TAGs are split into free fatty acid chains, and glycerol, 95% of the energy comes from FFAs, glycerol can go to glycolysis
- Free fatty acids instead are oxidized through Beta Oxidation, by breaking them into 2 carbon subunits to form Acetyl-CoA, NADH and FADH2 each cleavage
- B-Oxidation occurs in aerobic condition, in the mitochondria, and overproduction causes Ketosis
- **Regulation:** substrate availability: Lipases activated by glucagon and epinephrine (Starved) and inhibited by insulin (Fed), Malonyl-CoA inhibits CPT1 (FED) and Acetyl-CoA carboxylase inhibited by FFA concentration (Starved) so CPT1 Active
- **Steps of B-Oxidation:**
  1. **Activation:** of fatty acids by esterification with CoASH, forming fatty-acyl-CoA by Acyl-CoA synthase, using ATP→AMP, highly exergonic
  2. **Membrane Transport**: Rate limiting step, **Carnitine shuttle** replaces the CoASH via Carnitine-Acyl-Transferase, Carnitine enters mitochondrial matrix bound to Acyl, reforming Acyl-CoA by same enzyme, Carnitine leaves Mitochondrial Matrix

3. **<u>Carbon Backbone reduction Cycle</u>**: first 3 reactions create a carbonyl group on the B carbon, 4th cleaves the 'Beta-Keto-Ester' producing an Acetyl-CoA and a fatty acid with 2 less carbons:
    1. <u>Dehydrogenation:</u> Generating FADH2, forming a C-C double bond → Enoyl-CoA formed
    2. <u>Hydration</u>: Removal of double bond, addition of H2O → Hydroxy-Acyl-CoA
    3. <u>Dehydrogenation:</u> Generates NADH, 2 H from B-Carbon removed → Keto-Acyl-CoA
    4. <u>Carbon-Carbon Cleavage</u>: Acetyl-CoA cleaved off by Thiolase Enzyme

Strange cases of B-Oxidation

- Odd carbon number Fatty Acids end with a 3C chain-CoA, converted to succinyl-CoA with vitamin B12, only odd chain can contribute to gluconeogenesis in some way
    - Proponyl-CoA, carboxylase → Methylmalonyl-CoA Mutase → Succinyl-CoA
- Monounsaturated FAs are converted from a Cis → Trans bond by Enoyl-CoA Isomerase
- Polyunsaturated, usually requiring reductase enzymes to remove double bonds
- Peroxisomal beta oxidation is for very long branched FAs, uses Acyl-CoA Oxidase and forms peroxide from FADH2
- Albumin transports FFA from adipose to other tissues

Ketone Bodies

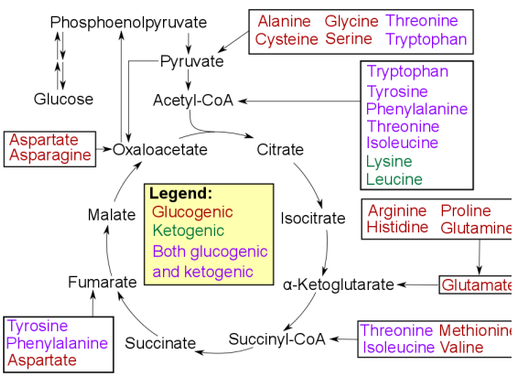
- Ketogenesis activated when OAA scares, result of excess B-Oxidation and the accumulation of Acetyl-CoA
- Mainly happens in the liver but cannot be used there, ketone bodies can reach brain or muscles
- Acetyl-CoA → Acetoacetyl-CoA → HMG-CoA → Acetoacetate(4C) → Acetone(3C) or B-Hydroxybutyrate (4)
- Keto Thiolase → HMG-CoA synthase → HMG-CoA Lyase → Hydroxybutyrate dehydrogenase
- Ketogenesis is regulated in the liver, by Carnitine transferase (CPT-1):
    - Malonyl-CoA is formed in FED state, inhibits CPT-1, no FA enter mitochondria
    - Acetyl-CoA carboxylase inhibited in STARVED state, Malonyl-CoA not synthesized, FA enter
- FFAs are mobilized from adipose tissue, but regulation happens in liver
- Acetyl-CoA from B-Oxidation will either enter Krebs, or produce ketone bodies

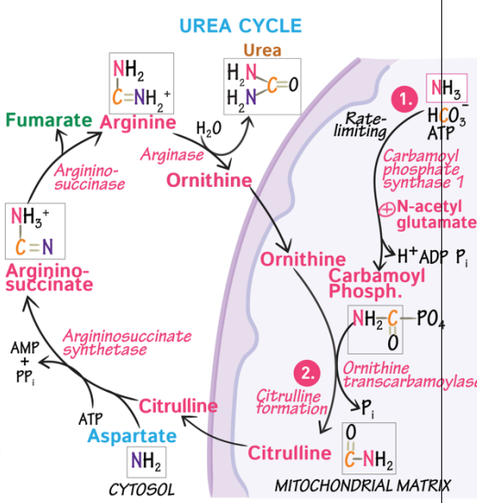
Protein degradation

- Endopeptidases in the stomach cleave polypeptides into smaller pieces, exopeptidases into di/tri peptides
- Peptidases can use acid, serine or cystine active sites, examples are Pepsin, Trypsin, Chymotrypsin
- Acidic Stomach acid promotes protein denaturation formed by HCl signaled by Gastrin
- Secretin signals the release of bicarbonate to neutralize stomach acids in small intestine
- Small intestine, more proteases act, di/tri peptides enter enterocytic cells via carrier mediated transport
    - Neutral, Aromatic, Basic, Acidic amino acids have different symports
- Di/Tri peptides are hydrolyzed in the enterocytes, and free amino acids exit via portal to liver
- Most amino acids are transaminated into alanine, Alanine is major Nitrogen carrier
- Amino acids can be ketogenic (converted to Acetyl-CoA) or glucogenic (pyruvate, or Krebs intermediate)
- Amino acid catabolism consists of: Deamination (Removal of NH4) Transamination (Moved to another) and oxidative decarboxylation (-COO removed)
- Transaminases equilibrate amino groups among <u>available a-keto acids</u>, N must be obtained from the diet
- Glutamine is formed from glutamate + NH4, leaving the amino group in the liver forming a-keto-glutarate
    - This reaction is called **oxidative deamination**
- This causes a buildup of Ammonia (NH3), or depletion of essential Krebs intermediate

Urea Cycle

- Occurs in the liver, using NH3 deposited by Glutamine and HCO to form **Carbamoyl Phosphate**
    - Carboxyphosphate + NH4 → Carbamoyl phosphate, ammonia recapturing
- The two nitrogen come from Carbamoyl Phosphate, and Aspartate (Transamination of OAA)
- Arginosuccinate connects Urea and Krebs, converted





to Arginine (Urea), and Fumarate (Krebs)

Regulation: Short by arginine concentrations, increase Carbamoyl Phosphate or Long by diet increasing transcription