

Glycolysis I

- First studied metabolic pathway
- Most of the enzyme studied are principles of carbohydrate metabolism
- 10 enzymatic reactions convert glucose \rightarrow 2 pyruvate
 - At the same time
 - $2 \text{ adp} \rightarrow 2 \text{ atp}$ net gain 2 atp
 - $2 \text{ nad}^+ \rightarrow \text{reduced nadh}$
- Total sum of energy: 2 ATP 2 NADH
- Glucose reduced over the course reduced form to oxidized form and the reducing potential transferred to NAD^+
- Preparatory phase- preparing molecule of glucose to extract energy for payoff phase
- Cost energy

- Molecule of glucose \rightarrow glucose 6 phosphate takes it from ATP
- Fructose 6-phosphate put another ATP \rightarrow Fructose 1,6-Bisphosphate
- Spends 2 ATP gets back 4
- Making lemonade

- Start with glucose
- Aldose sugar
- Take glucose and phosphorylate it add it to C6 GLUCOSE
 - By phosphorylating it, the glucose becomes traps in cell
 - No longer escape \rightarrow can perform glycolysis
- Isomerization-glucose aldehyde \rightarrow fructose 6 phosphate ketose
 - Carbonyl at c1 \rightarrow Carbonyl at C2
 - Take this glucose of
 - Why
 - At some point (6 sugar) 2 3 carbon break carbon- c bond to do that, place carbonyl to bond you wish to break. Moving this carbonyl from C1 \rightarrow C2 promote the carbon breakage at a later point.
- From fructose 6-phosphate \rightarrow fructose 1,6 bisphosphate
 - End goal is to get 2 3-carbons. At F6P, only one end has phosphate group. When it breaks, only one of 2 of the 3-carbon will get phosphate. That means, one of them will leave cell if not phosphorylated. So you spend another ATP. 2nd phosphorylation of the hydroxyl at C1 position
- Fructose 1,6-bisphosphate \rightarrow 2 3-carbons phosphorylated triose
 - Now you can break it in half, the carbonyl at the C2 position facilitates that to happen.
 - Fed into payoff stage
 - Locked in cell bc the phosphate groups.

- All glycolysis intermediates are phosphorylated

- Not depart the cell you don't want them to diffuse out of cell -> waste of energy
 - Glucose are imported through transporter because **they are no transporter for phosphorylated sugars**
 - **Conserves metabolic energy**- only happens because we couples it to hydrolysis ATP -> endergonic
 - The phosphate by forming covalent bond -> high energy sugar which can give energy back at a later step-> payoff step
 - Energy is when it is hydrolysis
 - Having phosphate group facilitate enzyme to bind to
 - Significant binding
 - Provide power for catalysis
- 1: Take glucose and convert to glucose 6-phosphate
 - Placing it on hydroxyl at c6.
 - Comes from atp
 - The same time take the phosphate group off ADP
 - Hexokinase enzyme that catalysis this step
 - priming - using ATP step
 - Priming it by coating it with a phosphate
 - Exergonic -16.7 kJ/mol
 - However adding phosphate is endergonic reaction
 - It is only because we coupled it with ATP hydrolysis that it is spontaneous provide free energy for it to happen.
 - Hexokinase - induced fit binding
 - Two domain protein
 - One binds to glucose one to atp
 - When glucose is absent (fasting), to bind to hexatose
 - Inactive conformation
 - Upon binding of glucose domain close brings atp and glucose to close proximity. Binding of glucose affect conformation of change
 - Take atp close proximity to glucose so it can be phosphorylated
 - No glucose around no glycolysis.
 - Only spending ATP when the glucose is around participating glycolysis
 - at active form ATP may be in a position to react with water to take the place of glucose.
 - Only active when glucose around for glycolysis
 - Isozyme: different enzymes that catalyze the same reaction 4 that does the same job
 - Hexokinase i in myocytes kM for glucose 0.2 mM.
 - Hexokinase iv (glucokinase) only in hepatocytes (10mM)
 - Y axis- initial rate/ v max
 - Fraction of maximal energy achieved at y axis at different glucose concentration

- Hexokinase i -energy goes up very quickly as glucose increase
- Even low concentration, it goes to max activity
- Hexokinase in muscle cell. Constantly running at fast rate, burning ATP not to be bothered by glucose level.
- Liver not an energy production organ but energy storage.
 - Glucose -(fasting) - glucokinase not to be competing with hexokinase. Use it for muscle not for liver.
 - Glucose high- converts to glucose 6-phosphate because you have more than you need for muscles.
 - As glucose levels increase, more glucose is stored away in the hepatocytes.
 - Won't max out will continue to store glucose even if you do something stupid (more ice cream).
 - Will respond to a whole range of glucose concentration.
- 2: Glucose-6-phosphate to Fructose-6-phosphate
 - Isomerization moving the carbonyl to c2 position by opening and closing the ring.
 - Enzyme: phosphohexose isomerase
 - $\Delta G = 1.7 \text{ kJ/mol}$
 - Binding energy opens
 - Base present by active site glutamate sidechain acts as base to extract H from C2 carbon.
 - Produce an enediol intermediate
 - Protonated glutamate gives carbon back to c2 carbon.
 - General acid catalysis by Glu facilitates formation of f6p.
 - Closes ring
- 3: Fructose-6-phosphate to Fructose-1,6-bisphosphate
 - ATP is hydrolyzed to ADP
 - Second priming step.
 - Phosphofructokinase-1
 - Committed step because
 - glucose -6p can be storage as glycogen in the liver
 - Or can be fed into pentose-p pathway
 - After it converts to fructose-6p, it is reversible, can go both directions because the ΔG is almost 0 ($\sim 2 \text{ kJ/mol}$)
 - However F6P to F1,6P it is destined for glycolysis.
 - This is the step you want to regulate because this is the step that is the bottleneck.
 - PFK controlling enzyme of glycolysis.
- PFK- allosteric regulator.
 - Tetrameric
 - All subunit contains active site can perform a reaction.
 - Each can bind to F6P and ATP.

- This pic is captured after the catalysis.
 - PFK at first conc of substrate
- Allosteric inhibitor #1- ATP
 - One at low ATP and high ATP, mk plot changes.
 - Low is regular kinda sigmodal
 - High shifts to right hand side.
 - Km increases in presence of high ATP.
 - High ATP you don't want more glycolysis
 - mediated through change by binding to allosteric site communicated to active site turns off binding site.
 - Since ATP concentration turns off pathway.
 - You don't need that much energy
 - Allosteric sites is where the allosteric regulators bind can change the conformation by binding to the active site so it can no longer bind to F6P.
- Citrate- Allosteric inhibitor #2
 - molecule involved in kreb cycle
 - high citrate- you won't be struggling for energy through glycolysis.
- Activators of PFK
 - ADP and AMP increase affinity for PFK
 - High adp means atp level is low you need more atp.
 - Fructose 2,6 bisphosphate increases enzymatic activity. (why?)
- 4: Cleavage of fructose 1,6-bisphosphate
 - **Make or break C-C bond**
 - Split between Carbon $\frac{3}{4}$
 - Breaking of c-c bond generate trioses
 - One ketose triose (Dihydroxyacetone phosphate)
 - One aldose triose (glyceraldehyde 3-phosphate)
 - Delta G is 23.8 really endergonic no ATP available.
 - Conc of product and reactant large effect on delta G.
 - Highly reversible depending on how you manipulate conc.
 - Use enzyme aldolase
- 5: Interconversion of triose phosphatase
 - Dihydroxyacetone phosphate -> glyceraldehyde 3-phosphate
 - 2 different products
 - Any of them can participate can in payoff stage
 - Wasteful if only 50% participate
 - Therefore
 - Second isomerization
 - Dihydroxyacetone phosphate -> Glyceraldehyde 3-phosphate
 - Enzyme: triose phosphate isomerase
 - Mechanism is the same as before just reverse. G3P-> F3P

- $G=7.5 \text{ kJ}$
- 2 gly3pho
- Where did the carbon go?
- Fructose 1,6-bisphosphate carbon 1-6
 - 1,2,3 go to dihydroxyacetone phosphate and 4,5,6 goes to glyceraldehyde 3-phosphate
 - Through TPI- \rightarrow 2 glyce3phosp
 - Now its just 1,2,3
 - 4-3 These are the corresponding carbons to original F1,6,B now with the Gly3-p
 - 5-2
 - 6-1

Glycolysis II

- Make some ATP
- Payoff phase
- What happens to the pyruvate
- aldose → ketose add phosphates
- Split into 2 bc 2 glyceraldehyde
- Everything is times 2.
- Phosphate groups is transferred to ATP
- Net is 2 molecules of ATP.
- S
- 6: Oxidation of glyceraldehyde 3 phosphate → 1,3-bisphosphoglycerate
 - Single phosphate add another one
 - Done as an **oxidation reduction** not as a phosphate transfer
 - Reaction With inorganic phosphate
 - Result aldehyde is oxidized has phosphate group to get acyl phosphate
 - Highly energetic
 - Energy produced in oxidation of aldehyde
 - Oxidation reduction is conducted by dehydrogenases
 - Typically uses $\text{NAD}^+ \rightarrow \text{NADH}$
 - Reduced
 - Glyceraldehyde is oxidized.
 - NAD^+ is far below typical glucose
 - You want to regenerate NAD^+ back
- S
- Cysteine residue
 - Presence of NAD^+ decreases the pKa of thiol group S- form
 - Really good as nucleophile
 - Negative S attack the aldehyde C and oxygen anion formed.
 - When the O- comes back and H goes to NAD → NADH
 - NADH is released and replaced with NAD^+ and the phosphate group.
 - Phosphate attacks the ketone C and Cysteine S is released as LG.
 - 1,3 bisphosphoglycerate is release
 - Endergonic reaction: $\Delta G = 6.3 \text{ kJ/mol}$
- S
- 7: Phosphoryl transfer from 1,3-bisphosphoglycerate
 - Making ATP
 - Energy in acyl phosphate
 - Substrate to ADP → ATP
 - Enzyme: phosphoglycerate kinase
 - Product: 3-phosphoglycerate + ATP
 - First (2) ATP production cell.
 - 6/7 are coupled.

- Delta G: -18.8 kJ/mol very favorable
- S
- 8: 3-phosphoglycerate → 2-phosphoglycerate
- Right now we got 2 ATP: made even
- Phosphoryl group here will eventually get transfer but transfer from the C3 position is not energetically favorable more favorable at C2 position.
- Isomerization
- Catalyzed by phosphoglycerate mutase
- Active site: 2 highly conserved Histidine.
- Lower histidine
 - Base catalysis taking H off hydroxyl group → oxygen anion
 - Attacks phosphate present in phosphate in histidine.
- C3 Oxygen grabs H from protonated histidine.
- Histidine with a phosphate group takes phosphate group in the C3 position back.
- Enzyme back to normal now
- S
- 9: Dehydration of 2-phosphoglycerate
 - Dehydration or elimination a molecule of water
 - Reason: difficult to perform of phosphate to get ATP
 - By performing dehydration, make this phosphate into a better LG so it is easier to add it to ADP.
- S
- Mechanism
 - Lysine in the active site that is deprotonated.
 - 3 structure Enolase is tailor that at that pH, lysine is deprotonated at physiological pH
 - Adj is a glutamate protonated.
 - 2 Mg bound to enolase
 - 1) lysine- deprotonated acts as base pulls H off C to form double bond → enolate intermediate.
 - 2) enolate now you get two Oxygen anion and a double bond between two C.
 - High energy species.
 - 3) to stabilize it two mg 2+ they can interact with the negative stabilize the transition state, can occur quickly
 - Protonated glu (not its normal protonation state)
 - Enzyme has evolved that it maintain this form for catalysis.
 - Protonated glu act as acid and facilitates the elimination of -OH.
 - (By manipulating the surrounding environment, you can change the pka values.
 - Now you get phosphoenolpyruvate and release of water.
 - Now it is good at release ATP.
- Phosphoryl transfer from phosphoenolpyruvate
 - Same as ATP transfer before add phosphate group to ADP
 - Enzyme: pyruvate kinase.
 - Net: 2 ATPs

- Energetically favorable because
 - really good at giving up its phosphate group
 - Tautomerization of pyruvate in enol form quickly go to keto form
 - Stabilizes the molecule and the delta G is large.
- **NOT ARBITRARY STEPS.**
- S
- After glycolysis
- 2 NAPH + 2 ATP
- Important to regenerate NAD⁺ to allow glycolysis to regenerate
- In aerobic respiration, oxidative phosphorylation in mitochondria.
 - Pyruvate → lactate
 - Ketone becomes hydroxyl
- In anaerobic respiration, oxidative can't occur mitochondria useless. Glycolysis only way.
- Alternative to aerobic is fermentation
 - Turns pyruvate → lactate
 - Reduced
- S
- Yeast ferment glucose to ethanol
- Rather than converting it to lactate, it converts to ethanol
- Pyruvate → (pyruvate decarboxylase) acetaldehyde → (alcohol dehydrogenase) ethanol
 - Bread to raise bubbles in champagne.
- Cofactor for pyruvate decarboxylase is TPP (thiamine pyrophosphate)
- S
- TPP carries aldehyde group
- Derived from vitamin B-1
- Active part is the C of the thiazolium ring
 - It will be deprotonated due to delocalization provided by ring structure
 - Negative on carbon pyruvate form of
 - TPP pyruvate attached to it
 - Electron sink all the electron pulled down → different groups on the substrate become better LG → loss of carboxylic group (for example)
- Warburg effect can be used to detect cancers.
- Can't perform aerobic respiration only glycolysis
- That means a lot of glucose
- Oxidative phosphorylation more efficient less glucose
- Tumor proliferate environment is anaerobic.
- Very reliant on glycolysis use this as early symptoms as early tumor.
- Tumor higher uptake in glucose burn more glucose in the surrounding environment.
- How to detect early tumors?
 - Take this glucose derivative with fluorine isotope.
 - When you convert it from glucose → glucose 6 phosphate (FdG → 6-phospho-FdG)
 - This won't be able to further turned into F6P.
 - Place them in PET scanner and scan for the decay of fluorine 18.

- Tissue that take glucose for glycolysis. Increase glucose uptake
 - Can infer that there is a tumor proliferation.

Gluconeogenesis

- Pathway that exist when glucose levels are low
- To create new molecules of glucose
- Under starvation
- Need it to power oxidative phosphorylation
- How does it compare to glycolysis
- Share same enzymes
- Some are like glycolysis- some not (bypass reaction)
- glycolysis and gluconeogenesis both have regulations.

- Gluconeogenesis creates glucose from non-carbohydrate sources.
 - Liver storage of glucose → start releasing glucose back to bloodstream
 - When glucose is low might be endanger of stopping glycolysis stopping energy production
 - Continue not to eat, deplete the storage of glucose.
 - This is when gluconeogenesis starts to happen.
 - Taking biomolecule feeding them into a pathway to get glucose to power glycolysis
 - Brain is bright neural cells rely heavily on glycolysis.
 - Many ways to feed into gluconeogenesis.
 - Pyruvate or lactate or amino acids to create glucose.
 - Only under extreme conditions
 - Will find that muscle mass is depleted if you keep starving because all the protein in it is used to feed into gluconeogenesis.
 - In mammals, cannot take fatty acid into gluconeogenesis.

- Gluconeogenesis is not glycolysis in reverse
 - Seems like the reverse of glycolysis but not really.
 - Many shared steps
 - Some steps during glycolysis are deemed as irreversible large negative delta G
 - Thermodynamically irreversible cannot just add more stuff.
 - Cliffs not go back up.
 - Solution: path that go back up. Alternative paths are bypass
 - 3 bypass reactions
 - Pyruvate +ATP-> Phosphoenolpyruvate+ADP
 - F1,6bis+ADP->f6p+atp
 - Glucose 6 phosphate ADP-> Glucose+ATP

- Irreversible steps of glycolysis require by-pass reactions
 - Different delta G values:
 - left hand: standard
 - right: exist within cell
 - Even reactions that have a large delta G, in the cell they are relatively close to 0.

- So if there is a large reactant conc than product than you can manipulate it to go forward
 - Likewise because it is close to 0 it is fairly reversibly so you can manipulate product concentration so it would go the other way
 - Reverse same enzyme to catalyze it.
 - Large negative G then we have to use the bypass reactions.
- Conversion of pyruvate to PEP is the first by-pass reaction
 - First bypass reaction
 - Making pyruvate from PEP
 - Pyruvate starts off in cytosol → mitochondria → back to cytosol for gluconeogenesis
 - Pyruvate goes to mitochondria (anyways will bc for krebs cycle)
 - When in mitochondria, **pyruvate reacts with bicarbonate → carboxylation reaction take carboxylate and add it to methyl group of pyruvate**
 - enzyme: pyruvate carboxylase
 - Produces oxaloacetate
 - Cost a molecule of ATP: $\text{Atp} \rightarrow \text{adp}$
 - Key feature cofactor: biotin vitamin b7 (tpp vit b1)
- Pyruvate decarboxylase requires a biotin cofactor
 - Pyruvate decarboxylase has 2 active sites. Two separate reactions
 - Function: Transfer of reactant from one active site to another is mediated by biotin cofactor
 - Active site 1 is activated by bicarbonate molecule
 - Bicarbonate is converted into CO_2
 - carboxylate group is added to biotin cofactor
 - After that, biotin swing from active site 1 → 2
 - Bc lysine is flexible
 - Pyruvate is waiting in active site 2
 - Second carboxylate is released in active site 2
 - CO_2 reacts with pyruvate to form oxaloacetate
 - If pyruvate was active in 1, it would interfere with the activation of bicarbonate, which allow to turn into carboxylate group.
 - Because biotin takes the intermediate to the other active site.
- Oxaloacetate cannot be transported from the mitochondrion.
- After it turns into oxaloacetate, it is ready to be transported out of mitochondria → cytosol
 - Problem can't because no transporter for it out of mitochondria.
 - Solution turn into malate have a transport
 - Take Oxaloacetate + $\text{NADH} \rightarrow \text{Malate} + \text{NAD}^+$
 - After it is transported out of the mitochondria
 - Turns it back into oxaloacetate

- Once oxaloacetate is out then it can be converted to PEP
 - Needs to be phosphorylated → Gets it from GTP
 - Carbonyl group will attack phosphate on GTP → form covalent bond between phosphate group and carbonyl get phosphorylated form
 - Decarboxylation of carboxylated group → CO₂
 - Formation of PEP
 - Enzyme of PEP carboxykinase
 - Standard $\Delta G = 0.9$ meh but exergonic in cell -25 almost irreversibly
 - Why is it so favorably?
 - PEP is a high energy product (wanted in other pathway) so it is always low so this forward reaction will always be favorable.
 - Cost a molecule of ATP and GTP. not an energetic proficient process
 - Why does this reaction proceed through the mitochondria?
 - Why not just have an enzyme in the cytosol that does this? Because **NADH**
 - Because it consumes NADH → NAD⁺
 - If it were to take place in cytosol, NADH would be consumed so fast and it is valuable in later step → if you deplete it then later step than you won't have anymore NADH.
 - You can deplete NADH in mitochondria bc [NADH] is high compared to cytosol
 - [NADH] in mitochondria doesn't matter as much. You can still have leftover for the rest of the reaction.
 - MOST IMPORTANTLY, Conversion of malate back to oxaloacetate in cytosol, Malate + nad⁺ → nadh + oxaloacetate
 - Not depleting [nadh] in cytosol but also producing nadh.
 - So switch from ox to ma to ox help shuttle nadh and you get 1 back so YAY!
- Alternative pathway uses lactate as the starting point
- Other types of gluconeogenesis feed into gluconeogenesis
 - In cytosol, lactate can be converted into pyruvate
 - Can be a source (reactant of gluconeogenesis)
 - enzyme: lactate dehydrogenase.
 - Lactate + NAD⁺ → Pyruvate + NADH + H⁺
 - Lactate to pyruvate → supply NADH
 - Pyruvate shuttled into mitochondria
 - Undergoes carboxylation of pyruvate to produce oxaloacetate.
 - Oxaloacetate → PEP
 - Lactate to pyruvate directly to PEP in mitochondria.
 - Why doesn't go through the whole malate shenanigans?
 - Because the whole point of that is to generate extra NADHs but here, we already generated a shit of NADH in the beginning.
 - How does it know control is done transcriptionally. Large amount of whatever.

- Bypass reaction 2: F1,6Bisphosphate → F6P
 - Pfk1 was heavily regulated.
 - Large negative G is needed bypass reactive
 - Another enzyme FBPase-1
 - Kinases are enzyme takes phosphoryl group from ATP and transfer it to substrate
 - Phosphorylases are the oppose take substrate with phosphoryl group and remove it.
 - Hydrolysis removing a phosphate
 - Both happening at the same time: hydrolysis of ATP. Bleeding ATP slowly.
 - $ATP + H_2O \rightarrow ADP + P_i$
 - FBPase heavily regulated.
 - AMP is allosteric inhibitor of FBPase-1

- F2,6bisphosphate is an allosteric regulator of PFK-1 and FBPase-1
 - Very heavily regulated because it will define if glycolysis or gluconeogenesis is happening in the cell.
 - 2,6 different position
 - Does not participate in either reaction.
 - Regulate both pathways.
 - Absence of F26P → pfk activity is extremely low
 - Added in km shift to left
 - Allosteric activator of
 - Absence of F26BP high affinity. Presence km shift to the right.

- Conc of f26bp is v important formation of this is very important.
 - Conc is high means glycolysis, low means gluconeogenesis
 - Formation if very important is to regulate the balance
 - Same enzyme that makes and destroy f26bp
 - 2 catalytic activity
 - Single protein different domains that we give a different functions
 - Makes the $F6P \rightarrow f26bp$ is PFK2 (active form) and FBPase2 (inactive form)
 - $F26BP \rightarrow F6P$
 - Single protein is controlling all of this
 - That switch between either state is by phosphorylation
 - Existed in 2 states: phosphorylated or dephosphorylated state
 - **Phosphorylated (yes phosphoryl group) → pfk (turn off) fbpase (turned on)**
 - When phop → start making f6p and breaking down f26bp
 - Decrease f26bp conc → turned off glycolysis turn on gly
 - **Dephosphorylated (no phosphoryl group) → pfk (turn on) fbpase (turn off)**
 - Stimulate glycolysis
 - Phosphorylation states are under hormonal control.
 - 2 hormonal determine phosphorylation
 - Insulin

- turns on protein phosphatase, which will dephosphorylate bifunctional enzyme (bye bye phosphate group)
 - **turn on PFK-2 turns off FBPase-2** → stimulates glycolysis.
- Glucagon turns on
 - turns on protein kinase, which will phosphorylate bifunctional enzyme (c'mon phosphate group)
 - **turn off PFK-2 turns on FBPase-2** → stimulates gluconeogenesis
- Summary: Insulin or glucagon influence phosphorylation state of enzyme change, which dictates which activity is going to happen.
- G6P → glucose is the 3rd bypass
 - $G6P + H_2O \rightarrow \text{Glucose} + P_i$
 - enzyme : glucose 6-phosphatase
 - Hydrolysis of phosphate group to produce glucose.
 - You don't want reverse reaction happening the same time takin
 - Most of the regulation is controlled downstream via the F26BP but there is additional regulations
 - Sequestration separate things regulation when you separate enzyme with each other.
 - Glucose 6 phosphatase in the lumen transported to the lumen of er
 - Separate 2 enzymes in two different locations.
 - Glucose 6-phosphatase is located in the lumen of the ER so it is sequestered away in the ER
 - Under conditions of gluconeogenesis, g6p will be transported to the lumen of the ER where it can access this enzyme and turned into glucose and transported back out of the cytosol and transported in the capillary .
- Gluconeogenesis requires more energy than glycolysis
 - If reverse glycolysis than it would be 2ATP but no
 - Cost of gluconeogenesis 4 ATP, GTP, 2NADH. Not energy sufficient
- Any molecule capable to be transported into kreb cycle than that can in principle be feed into kreb cycle.
 -