Gluconeogenesis

• The biosynthesis of glucose

So far we have studied the breakdown of glucose. What if we need to make glucose?

Why would we need to make it?
1. To maintain our blood glucose levels (prevent hypoglycemia)
2. Shift sugar/energy to important body parts (brain and muscles)

Brain is top priority!
1. Needs large amounts of energy
2. Cannot store energy (very little glycogen storage)
3. Not sensitive to insulin regulation
4. Must have glucose for energy! Will not adapt to using ketone bodies (fat) for energy until dire conditions arise (weeks of fasting)

• Process by which glucose is made from non-carbohydrates
  1. Lactate
  2. Pyruvate
  3. Amino acids (particularly Ala)
  4. Glycerol (from triacylglyceride hydrolysis) enters as dihydroxyacetone phosphate
  5. Acetate

• Major sites of gluconeogenesis:
  1. Liver (90%)
  2. Kidney (10%)

Net reaction:

\[
2 \text{pyruvate} + 2 \text{NADH} + 4 \text{ATP} + 2 \text{GTP} + 6 \text{H}_2\text{O} + 2 \text{H}^+ \rightarrow \text{Glucose} + 2 \text{NAD}^+ + 4 \text{ADP} + 2 \text{GDP} + 6 \text{P}_i
\]
Gluconeogenesis is not simply the reversal of glycolysis!!!!

Gluconeogenesis:
Adds 4 new steps to circumvent the irreversible (& highly regulated) steps of glycolysis

1. _________________
2. _________________
3. _________________

Gluconeogenesis occurs ONLY when these enzymes are inactive.

This prevents ineffective cycles
• Reactions take place in the cytosol except for:
  1. Pyruvate carboxylase (mitochondria)
  2. Glucose-6-phosphatase (ER)

• Again we see the importance of compartmentalization:
  Prevents direct competition of gluconeogenesis and glycolysis

### Table 15.4
The reactions of gluconeogenesis beginning with pyruvate

<table>
<thead>
<tr>
<th>Number</th>
<th>Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pyruvate + CO₂ + ATP → oxaloacetate + ADP + P_i</td>
</tr>
<tr>
<td>2</td>
<td>Oxaloacetate + GTP → phosphoenolpyruvate + CO₂ + GDP</td>
</tr>
<tr>
<td>3</td>
<td>Phosphoenolpyruvate + H₂O → 2-phosphoglycerate</td>
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<tr>
<td>4</td>
<td>2-Phosphoglycerate → 3-phosphoglycerate</td>
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<tr>
<td>5</td>
<td>3-Phosphoglycerate + ATP → 1,3-bisphosphoglycerate + ADP</td>
</tr>
<tr>
<td>6</td>
<td>1,3-Bisphosphoglycerate + NADH + H⁺ → glyceraldehyde-3-phosphate + NAD⁺ + P_i</td>
</tr>
<tr>
<td>7</td>
<td>Glyceraldehyde-3-phosphate → dihydroxyacetone phosphate</td>
</tr>
<tr>
<td>8</td>
<td>Glycerol-3-phosphate + dihydroxyacetone phosphate → fructose-1,6-bisphosphate</td>
</tr>
<tr>
<td>9</td>
<td>Fructose-1,6-diphosphate + H₂O → fructose-6-phosphate + P_i</td>
</tr>
<tr>
<td>10</td>
<td>Fructose-6-phosphate → glucose-6-phosphate</td>
</tr>
<tr>
<td>11</td>
<td>Glucose-6-phosphate + H₂O → glucose + P_i</td>
</tr>
</tbody>
</table>

Gluconeogenesis: Steps 1 & 2

These 2 steps are required to bypass pyruvate kinase

Requires the cleavage of 2 phosphoanhydride bonds

Proceeds through an oxaloacetate intermediate

Gluconeogenesis: Step 1

• Pyruvate carboxylase (enzyme)

  - Carboxylation reaction
  - Irreversible
  - Synthesis of C-C bond; requires energy

![Energy input:](image)
Gluconeogenesis: The forgotten step!

- In order for oxaloacetate to leave the mitochondria, it must be reduced to malate.
- Malate can be transported out of mitochondria (refer to figure 14.19)
- Malate is then reoxidized to oxaloacetate in the cytosol

Gluconeogenesis: Step 2

- PEPCK is enzyme
- Decarboxylation reaction
- Group transfer reaction (phosphoryl transfer)

Energy input:

Gluconeogenesis: Steps 3-8

The reverse of the reactions we discussed with glycolysis

Enzymes are the same

None of these steps is regulated

None of these steps require energy

Step 8 produces fructose-1,6-bisphosphate from triose phosphates
Gluconeogenesis: Step 9

- Reverse phosphofructokinase
- This reaction is irreversible (ΔG = -16 kJ/mol)
- Hydrolysis reaction

Gluconeogenesis: Step 10

Reverse of glycolysis

Gluconeogenesis: Another forgotten step!

- We need to transport glucose-6-P from the cytoplasm into the ER
- A transporter known as GLUT7 transports Glc-6-P
- GLUT7 is only found in liver, kidney, pancreas, and small intestine

Gluconeogenesis: Step 11

- Reverses hexokinase
- Irreversible reaction (ΔG = -13.8 kJ/mol)
- Hydrolysis reaction

Gluconeogenesis: Last forgotten step

Glucose diffuses through ER membrane into blood stream

Gluconeogenesis: Regulation

- Which pathway is on? Glycolysis or gluconeogenesis?
  - Substrate cycling: If there are two opposing enzymes, altering 1 enzyme, changes the flux through the opposing enzyme. (1 off, 1 on)
• Which pathway is on? Glycolysis or gluconeogenesis?

• Energy levels (ATP v AMP) dictate!
  -_____ more prevalent leads to gluconeogenesis
  -_____ more prevalent leads glycolysis
  -_____ inhibits, _____ stimulates phosphofructokinase (glycolysis)
  -A decrease in glycolysis increases gluconeogenesis (substrate cycling)

  -_____ inhibits fructose-1,6-bisphosphatase
  -Fructose-2,6-bisphosphate inhibits fructose-1,6-bisphosphatase

![Diagram of glycolysis and gluconeogenesis pathways](image)

• Acetyl CoA is an allosteric activator of pyruvate decarboxylase
  -acetyl CoA signals energy is prevalent
  -Acetyl CoA can bind to Pyruvate Carboxylase and activates the enzyme!
Now we know how to breakdown AND synthesize glucose

Occasionally, glycolysis and gluconeogenesis must occur simultaneously

Cori Cycle

- In muscle: Occurs when our energy needs deplete our oxygen supply; we then switch from aerobic metabolism to anaerobic metabolism.
  - exercising

- If you are intensely exercising, your muscles are:
  - Using up glucose through glycolysis
  - Using up NAD\(^+\) through glycolysis
  - Producing pyruvate, which cannot be further oxidized because there is no oxygen
    - Pyruvate takes the anaerobic pathway, and is converted to lactose
    - This leads to the reduction of NADH to NAD\(^+\), allowing glycolysis to continue.
    - This also leads to a build up of lactate

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Glucose</th>
<th>ATP</th>
<th>NAD+</th>
<th>NADH</th>
<th>pyruvate</th>
<th>lactate</th>
</tr>
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</table>

- The lactate that builds up can lead to a decrease in blood pH (lactic acidosis)
  To prevent this:
  - Lactate is transported (through the blood stream) to the liver
  - In the liver (where there is O\(_2\)), it is oxidized back to pyruvate
  - Pyruvate is converted, via gluconeogenesis, back to glucose

- Our glucose supply is decreasing in the muscle
  - Glucose from liver is transported back to the muscle, through the blood stream

**Most important aspects of Cori Cycle:**
1. Removes lactate from muscle, preventing decreases in pH
2. Allows for generation of glucose, thus allowing our muscles to have energy

see also fig 12.2 of your book

Chemistry C483 Fall 2009 Prof Jill Paterson 36-7
Glycogen Metabolism

Where do we get the glucose that we use in glycolysis?

1. From our diet, where it directly enters our blood stream and enters appropriate cells
   - this is our normal source of glucose

2. From glycogen stores
   - polymer of glycogen (remember lecture notes 17 & 18?)
   - this is 1 of 2 energy stores
     - glycogen
     - fat
   - we degrade (use, mobilize) glycogen when glucose and ATP levels are ___
   - we synthesize (produce) glycogen when glucose and ATP levels are ______

glycogen metabolism: the balance & control between use and production

How do we make glycogen?

[Diagram of glycogen metabolism]
1. Need glucose-6-phosphate

Regulation at Step 3: phosphofructokinase-1 (PFK-1)

- Multiple things regulate PFK-1
  - ATP
  - AMP
  - ADP
  - Citrate
  - Fructose-2,6-bisphosphate

Synthesis of glucose-1-P

UDP-glucose

- UTP transfers UMP to phosphate on C-1
- forms UDP-glucose (2\textsuperscript{nd} P from glucose), and PPi (2 P from UTP)
Glycogen synthesis-linear

 enzyme: amylo-(1,4→1,6)-transglycosylase

Mechanism:
1. Removes 1 oligosaccharide of at least 6 glucose molecules in length from a non-reducing end.
2. Attaches this oligosaccharide to a glucose molecule via a $\alpha-(1\rightarrow6)$ linkage.
3. This linkage occurs at least 4 glucose molecules away from another branching site.
Glycogen v. fat for storage

While we generate more energy from our fat stores (see future lecture), there are advantages to storing energy in the form of glycogen.

1. Glycogen can be rapidly accessed in muscle for oxidation (fat cannot)
   - pluck off a glucose and it is ready to enter glycolysis

2. Glycogen does not need ______________ to initiate oxidation (fat needs __________ input prior to oxidation)
   - pluck off a glucose and it is ready to enter glycolysis

3. Glycogen can be used in the absence of ______________ (fat cannot)
   - ______________ metabolism

4. Glycogen produces glucose that maintains blood sugar levels (fat cannot be converted to ____________, so it cannot maintain blood sugar levels)

Glycogenolysis

• The process of removing a glucose molecule from glycogen is glycogenolysis

• Removal of a glucose (cleavage reaction) occurs via phosphorolysis:
  - the cleavage of a bond by Pi

• This is analogous to hydrolysis (like what occurs in saliva for breakdown of starch), but not quite as simple
What happens to glucose-1-phosphate?

\[
\text{phosphoglucomutase}
\]

Glu-1-P \quad \rightarrow \quad \text{Glu-6-P} \quad \rightarrow \quad \text{Glycolysis}

Glu-6-P enters glycolysis \underline{\text{before}} \text{ the hexokinase step.}

Since we produced Glu-6-P without using an ATP, but Glu-6-P produced by hexokinase uses an ATP, we have a higher NET ATP yield.

Glycolysis NET is \underline{\text{3}} \text{ ATP (instead of the normal \underline{\text{2}} NET ATP)}

(and still have 2 NADH)

Glycogen phosphorylase derived Glu-6-P

On your own:

1. Determine where Glu-6-P enters glycolysis

2. Do an energy count- do we get the same amount of energy as we do when we start with a glucose molecule?

3. Is regulation different from glucose?

How do we get glucoses near or at a branching point?

- Need glycogen debranching enzyme, which possesses two activities:
  1. 4-\(\alpha\)-glucanotransferase activity catalyzes the transfer of a trimer from a branch of the limit dextrin to a free end of the glycogen molecule.
     
     \[
     \text{glucose molecules can then be removed with glycogen phosphorylase}
     \]
  2. The amylo-1,6-glucosidase activity catalyzes hydrolytic release of the remaining \(\alpha-(1\rightarrow6)\)-linked glucose residue.
Debranching enzyme

Debranching enzyme yields LESS NET ATP than glycogen phosphorylase

- Debranching enzyme generates ___ glucose molecule
- This molecule enters glycolysis as _______________
- Thus our net ATP yield is ___ ATP
- This is ______ than the glucose-6-P that enters due to glycogen phosphorylase

On your own:
1. Determine where Glu-6-P enters glycolysis
2. Do an energy count- do we get the same amount of energy as when we start with a glucose molecule?
3. Is regulation different from glucose?

Regulation of glycogen metabolism

**Enzyme level**
- We have already discussed regulation by ATP and citrate in terms of increasing glucose-6-P production & hence glycogen production
- Glycogen phosphorylase (part of glycogen mobilization) is inhibited by ATP and glucose-6-P, while being activated by AMP.
Hormonally

- **Insulin**
  - When glucose levels are high (fed state)
  - Rate of glucose transport into cells via GLUT4
  - Rate of glycogen synthesis
  - A slow and steady regulation (maintains blood glucose levels)
  - Liver stores of glycogen
  - Muscle stores of glycogen

- **Glucagon**
  - When glucose levels are low (fasting state)
  - Rate of glycogen degradation
  - A slow and steady regulation (maintains blood glucose levels)
  - Liver stores of glycogen
  - Muscle stores of glycogen

- **Epinephrine**
  - When we are stimulated (fight or flight response)
  - Rate of glycogen degradation
  - Liver stores of glycogen
  - Muscle stores of glycogen

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Stimulation or increase</th>
<th>Inhibition or decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>transport into cells</td>
<td>Blood glucose levels</td>
</tr>
<tr>
<td>Glucose</td>
<td>Glycolysis</td>
<td>Gluconeogenesis</td>
</tr>
<tr>
<td>Glucose</td>
<td>Glycogen synthesis</td>
<td>Glycogen mobilization</td>
</tr>
<tr>
<td>Glucose</td>
<td>TAG synthesis</td>
<td>TAG breakdown</td>
</tr>
<tr>
<td>Blood glucose</td>
<td>levels</td>
<td>Glycogen synthesis</td>
</tr>
<tr>
<td>Glucose</td>
<td>Glycogen breakdown</td>
<td>Glycolysis</td>
</tr>
<tr>
<td>Glucose</td>
<td>cAMP production (liver)</td>
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</tr>
<tr>
<td>Blood glucose</td>
<td>levels</td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>cAMP production (muscle)</td>
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<td>TAG breakdown</td>
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</tr>
<tr>
<td>Glucose</td>
<td>Glycogen breakdown</td>
<td></td>
</tr>
<tr>
<td>Ethanol</td>
<td>metabolism</td>
<td></td>
</tr>
</tbody>
</table>

- **A carbohydrate**

- **Most ethanol metabolism occurs in the liver**
  - Some also in the stomach, kidneys, and bone marrow

- **Two enzymes involved:**
  1. Alcohol dehydrogenase (ADH)
  2. Aldehyde dehydrogenase (ALDH)
Ethanol consumption leads to production of an excess amount of NADH

This does not lead to energy production!

This leads to a large alteration in NAD+/NADH ratios

This large amount of NADH actually inhibits all NAD+ reactions (and hence metabolic pathways) because there is very little NAD+ around

Most notably we see decreases (or termination entirely) of fatty acid oxidation & gluconeogenesis

1. Inhibition of fatty acid oxidation (breakdown)
   - When there is a decrease in TAG breakdown, there is a stimulation of TAG synthesis; so our liver starts to synthesize MORE TAGs.

   - Increased level of TAG in liver

   - TAGs lead to fatty deposits in the liver; contribute to cirrhosis of the liver

2. Inhibition of gluconeogenesis
   - buildup of lactate (lactic acid) and can lead to acidosis (decrease in blood pH without symptoms)

   - Hypoglycemia

3. Acetaldehyde is VERY reactive!
   - forms adducts with proteins (reacts with NH₂ group)
   - forms adducts with various biomolecules with NH groups

   This can prevent proteins from functioning correctly