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Chapter 23

Gluconeogenesis
Glycogen Metabolism
Pentose Phosphate Pathway

Slide 2

Gluconeogenesis

- Humans use about 160 g of glucose per day, about 75% for the brain.
- Body fluids and glycogen stores supply only a little over a day's supply.
- In absence of dietary carbohydrate, the needed glucose must be made from non-carbohydrate precursors.
- That process is called **gluconeogenesis.**

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Gluconeogenesis, con't.

- Brain and muscle consume most of the glucose.
- Liver and kidney are the main sites of gluconeogenesis.
- Substrates include pyruvate, lactate, glycerol, most amino acids, and all TCA intermediates.
- Fatty acids cannot be converted to glucose in animals.
  - (They can in plants because of the glyoxalate cycle.)

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Gluconeogenesis, con't.

- Substrates include anything that can be converted to **phosphoenolpyruvate .**
- Many of the reactions are the same as those in glycolysis.
  - All glycolytic reactions which are near equilibrium can operate in both directions.
  - The three glycolytic reactions far from equilibrium (large -ΔG) must be bypassed.
  - A side by side comparison is shown in Fig 23.1.

Remember it is necessary for the pathways to differ in some respects, so that the overall ΔG can be negative in each direction. Usually the steps with large negative ΔG of one pathway are replaced in the reverse pathway with reactions that have a large negative ΔG in the opposite direction.

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Unique Reactions of Gluconeogenesis

- Recall that **pyruvate kinase**, though named in reverse, is not reversible and has a ΔG of –23 kJ/mol. (Table 19.1b)
- Reversal requires an input of **two** ATP equivalents, and hence **two** reactions.
  - Pyruvate carboxylase (Fig 23.2)
    - Recall the biotin prosthetic group.(Fig 23.3)
  - PEP Carboxykinase (Fig 23.6)

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Pyruvate Carboxlyase

- Already discussed as an **anaplerotic reaction.**
- Biotin prosthetic group communicates between a **biotin carboxylase** and a **transcarboxylase**, with **carboxybiotin** an intermediate. (Fig 23.4)
- Activated by acyl-CoA.
- Located in mitochondrial matrix.

Acyl-CoA activation, especially acetyl-CoA, regulates the fate of pyruvate. When acetyl-CoA is low, pyruvate is broken down by pyruvate dehydrogenase. High levels of acetyl-CoA signal the need for more OAA to run the citric acid cycle, or to be converted to glucose.

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PEP Carboxykinase

- Also mentioned as a possible anaplerotic reaction because it is reversible.
- GTP is utilized, but that is equivalent to ATP.
- Additonal GTP makes formation of PEP energetically favorable.
- In the cytoplasm in some tissues, in the mitochondrial matrix in others.
  - Presence in cytoplasm provides a means of exporting electrons from NADH. (Fig 23.5)

Because the oxaloacetate made in mitochondria cannot cross the mitochondrial membrane, it is reduced to malate, which can cross. Conversion of the cytoplasmic malate back to oxaloacetate in the cytoplasm produces the NADH needed for reduction of 1,3-bisphosphoglycerate.

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Unique Reactions of Gluconeogenesis, con't.

- The two additional steps bypassed are **hexokinase** and **phosphofructokinase**.
- The gluconeogenic enzymes are **phosphatases**, which simply hydrolyze the phosphate esters in an energetically favorable reaction.
  - Fructose-1,6-bisphosphatase (Fig 23.7)
  - Glucose-6-phosphatase (Fig 23.9)

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Glucose-6-phosphatase

- A membrane enzyme associated with the endoplasmic reticulum of liver and kidney.
- Hydrolysis probably occurs with release of free glucose into vesicles. (Fig. 23.8)
- Fusion of vesicles with cell membrane releases glucose into the blood.
- **Muscle and brain lack this enzyme.**

Glucose-6-phosphatase is actually used as a "marker" enzyme to measure the amount of endoplasmic reticulum present in sub cellular fractionation experiments.

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Regulation of Hexokinase and Glucose-6-phosphatase

- Hexokinase is inhibited by glucose-6-phosphate
- Glucose-6-phosphatase has a high Km for glucose-6-phosphate
- Therefore gluconeogenesis is favored by high concentrations of glucose-6-phosphate.

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Fructose-1,6-Bisphosphatase

- Allosterically regulated in a fashion **opposite** to phosphofructokinase.
  - Citrate stimulates.
  - AMP inhibits, ATP activates.
  - Fructose-2,6-bisphosphate (F-2,6-BP) inhibits. (Fig 23.12)
- High energy state (high ATP, citrate) stimulates the phosphatase and gluconeogensis.
- Low energy state (high AMP, low citrate) stimulates PFK and glycolysis.
- Hormonal regulation acts through F-2,6-BP.

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Overall Stoichiometry from Pyruvate

- Recall the stoichiometry of glycolysis was:

$$\text{Glucose} + 2\ \text{NAD} + 2\ \text{ADP} + 2\ P_i \rightarrow 2\ \text{pyruvate} + 2\ \text{NADH} + 2\ \text{ATP}$$

The stoichiometry of gluconeogenesis is:

$$2\ \text{pyruvate} + 2\ \text{NADH} + 6\ \text{ATP} \rightarrow \text{glucose} + 2\ \text{NAD} + 6\ \text{ADP} + 6\ P_i$$

(where ATP is equivalent to GTP)

- The difference of 4 ATP is sufficient to give the reverse reaction a negative ΔG.

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The Cori Cycle

- Vigorous exercise cause muscles to produce lactate.
  - (white muscle, low in mitochondria, is geared for rapid anaerobic glycolysis)
- The lactate is excreted into the blood and taken up by liver.
- Liver converts some of the lactate back to glucose.
- The glucose excreted by the liver can then be used by muscles.
- See Fig. 23.10

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Regulation of Gluconeogenesis

- Fig 23.11 summarizes the reciprocal regulation of glycolysis and gluconeogenesis.
- High energy status favors gluconeogenesis
- Low energy status favors glycolysis
- Acetyl-CoA influences the fate of pyruvate
- F-2,6-BP favors glycolysis and inhibits gluconeogenesis.

High acetyl-CoA inhibits favors pyruvate to oxaloacetate, either for increased TCA activity or increased gluconeogenesis. Low acetyl-CoA favors breakdown of PEP and pyruvate to form acetyl-CoA.

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Hormonal Regulation of F-2.6-Bisphosphatase

- F-2,6-BP made by PFK-2
- F-2,6-BP degraded by F-2,6-BPase
- Both activities are on the same protein
  - A **bifunctional enzyme** (Fig 23.13)
- Hormonal stimulation results in phosphorylation of this protein.
  - In **liver**, phosphorylation activates F-2,6-BPase, inhibits PFK-2. (stimulating gluconeogenesis)
  - In **muscle**, phosphorylation activates PFK-2, inhibits F-2,6-Bpase. (stimulating glycolysis)

We will discuss the mechanism of hormone activation a bit later when we discuss regulation of glycogen synthesis and breakdown.

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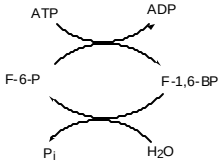
Futile Cycles

- If PFK-1 and F-1,6-Bpase are both active, they form a **futile cycle.**



Overall Reaction:

$$ATP + H_2O \longrightarrow ADP + P_i$$

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Regulation of the Futile Cycles

- Regulation is geared so that both enzymes should not be active at the same time.
- Both enzymes show allosteric behavior, with reciprocal responses to AMP, citrate, and F-2,6-BP.

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Substrate Cycles

- Why does muscle have F-1,6-BPase in the first place?
  - Muscle is not a gluconeogenic tissue.
  - It lacks glucose-6-phosphatase.
  - So what function does F-1,6-BPase serve?
- It has been suggested that a futile cycle actually occurs in muscle, but it is referred to as a **substrate cycle**.

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Substrate Cycles, con't.

- Anaerobic muscle needs to be able to quickly achieve a many fold stimulation of the glycolytic rate.
- Changes in allosteric effector concentrations may give only a few fold stimulation.
- But reciprocal changes in two opposing reactions provide an amplification of the signal.

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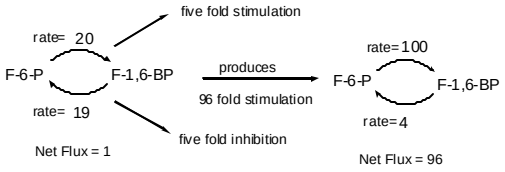
Substrate Cycles, con't.



- The price one pays for such control is the "wasteful" hydrolysis of ATP

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Glycogen Metabolism

- Recall glycogen structure
  - α-1,4 glucose polymer, α-1,6 branches.
  - One reducing end, many non-reducing ends.
- Dietary glycogen and starch are degraded by **amylases**
  - Alpha-amylase (saliva) is an *endoglyosidase*
    - (Fig 23.14)
  - Debranching enzyme and α-1,6-glycosidase needed for complete degradation.

The distinction between alpha and beta amylases is not on the configuration at the anomeric carbon. Beta-amylase is an exopeptidase in plants that cleaves disaccharide (maltose) units from the non-reducing ends of the glycogen chain.

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Glycogen Metabolism, con't.

- Tissue glycogen is degraded by **phosphorolysis** of the α-1,4 bonds. The enzyme is **phosphorylase**.
  - A cleavage by **phosphate** at the non-reducing end. (Fig 23.16)
- The reaction is reversible.
- Glucose-1-phosphate is the product.
- G-1-P is converted to G-6-P by the enzyme **phosphoglucomutase**.
- Debranching enzyme is needed for complete degradation.

There are many non-reducing ends on the glycogen on the glycogen polymer, so cleavage can occur at many sites on the same molecule at the same time.

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Glycogen Synthesis

- Originally thought to be phosphorylase acting in the reverse direction.
- Discovery of activation of sugars as nucleotide diphosphate derivates.
  - Uridine diphosphate glucose **(**Fig 23.17)
- Synthesis of UDP-glucose:

  UTP + G-1-P ¾ UDP-Glc + $P{\sim}P_i$
- Reaction is reversible, and named in reverse:

  UDP-glucose pyrophosphorylase

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Glycogen Synthesis, con't.

- **Glycogen Synthase** catalyzes the transfer of glucose from UDP-glc to the non-reducing ends of glycogen, forming an α-1,4 linkage.
  - Fig 23.19
- A **branching enzyme** transfers a 6-7 residue chain to the 6 position to form the branch points. (Fig 23.20).

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Control of Glycogen Metabolism

- Regulation of **phosphorylase** and **glycogen synthase** occurs at two levels.
  - Allosteric regulation by metabolites
    - Phosphorylase by AMP, ATP, G-6-P and glucose
    - Glycogen synthase by G-6-P
  - Hormonal regulation by covalent modification
    - Phosphorylation (by a protein kinase)
    - Dephosphorylation (by phosphoprotein phosphatase)
- See Chapter 15.5 for details.

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Glycogen Phosphorylase Regulation (Chapter 15.5)

- Phosphorylase is a dimer (Fig 15.18)
- It can exist in two conformational states.
  - An **R** state (active)
  - A **T** state (inactive)
- Non-phosphorylated form is in the T state.
- Phosphorylation (by **phosphorylase kinase**) shifts the equilibrium to the R state.

Remember the Monod-Wyman-Changeux model for allosteric regulations. Review it in section 15.4.

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Glycogen Phosphorylase Regulation, con't.

- The phosphorylated (active) form is called **phosphorylase a**
- The non-phosphorylated (inactive form) is called **phosphorylase b**
- **AMP** can shift phosphorylase b from the T conformation to the R conformation, activating the inactive enzyme when energy supply is low.
- See Fig 15.17 for a summary diagram.

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Glycogen Phosphorylase Regulation, con't.

- The phosphate groups of phosphorylase a are buried in the R conformation.
- In liver, glucose is an allosteric effector shifting the enzyme to the T conformation
- In the T conformation, **phosphoprotein phosphatase** can access the phosphate groups and hydrolyze them.

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Glycogen Phosphorylase Regulation, con't.

- The phosphatase therefore reverses the effect of the hormonal stimulation.
  - In liver, it is bound to phosphoryase a, and inactive until glucose shifts the conformation from R to T. Then the phosphatase is released and acts on a several different phosphorylated proteins, including glycogen synthase.
  - In muscle, the phosphatase is bound to a protein phosphatase inhibitor, itself regulated by phosphorylation-dephosphorylation. Insulin deactivates the inhibitor, activating the phosphatase.

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Glycogen Synthase Regulation

- Glycogen synthase also exists in two covalent modified forms:
  - Phosphorylated form is called the **D** (dependent) form (also called glycogen synthase b)
    - Allosterically activated by G-6-P
  - Non-phosphorylated form is called the **I** (independent) form (also called glycogen synthase a)
- Hormonally stimulated phosphorylation **inactivates** the synthase, while a phosphatase **activates** the synthase.

Therefore hormonal stimulation leads to activation of phosphorylase and deactivation of glycogen synthase, leading to glycogen breakdown. The enzymes are reciprocally regulated. Recall that hormonal stimulation also leads to phosphorylation of the PFK-2:F-2,6-BPase bifunctional enzyme with different effects in liver and muscle. Glycolysis is promoted in muscle, while gluconeogenesis is promoted in liver.

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Hormonal Stimulation of Protein Phosphorylation

- Hormonal stimulation of phosphorylase activity was the first case of a "second messenger" carrying the signal into the cell.
- The second messenger was identified as 3',5'-cyclic AMP by Earl Sutherland about 1967.
  - Adenyl cyclase makes cyclic AMP from ATP (Fig 34.2)
- First model was hormone binding to a receptor, stimulating adenyl cyclase, with cyclic AMP exerting its effects. (Fig 34.3)

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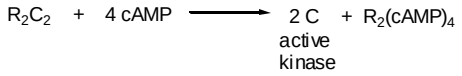
Cyclic AMP Signaling Pathway

- Now much more detail of the pathway is known.
- Cyclic AMP binds to the regulatory subunits of a **cyclic AMP dependant Protein Kinase** (**cAPK**), freeing the catalytic subunit which then phosphorylates cellular targets (for ex. Fig 15.19)



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Cyclic AMP Signaling Pathway, con't.

- cAPK phosphorylates a variety of cellular proteins.
  - **Phosphorylase kinase** is activated by phosphorylation.
  - **Glycogen synthase** phosphorylated both by cAPK and by phosphorylase kinase.
    - (Deactivated by phosphorylation.)
  - **PFK-2/F-2,6-Bpase** (bifunctional enzyme)
    - (Remember muscle and liver respond differently)
  - **Protein phosphatase Inhibitor** (activated in muscle by phosphorylation).

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Cyclic AMP Signaling Pathway, con't.

- A **G protein** mediates the signal between the hormone receptor and adenyl cyclase.
  - Its inactive form is bound to **GDP** and two protein subunits (β and γ)
  - When activated, **GTP** replaces the **GDP**, and the subunits dissociate.
  - It then deactivates itself by hydrolyzing the GTP.
  - See Fig. 15.21 and 34.5

G in G protein stands for GTP-binding protein.

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Other Cyclic AMP and Second Messenger Pathways

- Since its initial discovery, cAMP has been implicated in many other signaling pathways.
- Other second messengers have been discovered.
  - (Ca^{2+}, inositol triphosphate, diglyceride, for ex.)
- Phosphorylation targets can differ in different cells.
  - (hormone sensitive lipase in adipose tissue, for ex.)

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Other cAMP and Second Messenger Pathways, con't.

- A single hormone can have different receptor types, with different effects.
  - (α_1, α_2, β_1, and β_2 adrenergic receptors, for ex.)
- G proteins can be stimulatory in some cases, inhibitory in others.
  - (See Fig. 34.6)
- Different hormones can affect the same or similar pathways.
  - (glucagon and epinephrine, for ex.)

Figure 34.6 shows an α_2 receptor activating an inhibitory G protein, and a β_2 receptor activating a stimulatory G protein. Drugs have been developed that can target specific receptor types, some acting as **agonists** (mimicking the hormone), some acting as **antagonists**, blocking the action of the hormone.

Chapter 34 attempts to organize the ever-increasingly complex signaling mechanisms. We may not get a chance to get into details of all of them, but you should read the chapter for background information.

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Effects of Insulin

- Made by the alpha cells of the pancreas.
- Receptor is a tyrosine kinase.
  - (Chapter 34, page S-24)
- Multiple effects, one of which is to enable glucose uptake by tissues and to lower blood glucose. Gluconeogenesis is inhibited.
  - (See Fig 23.22)
- Part of the effect on glycogen regulation is the activation of **phosphoprotein phosphatase**, which reverses the effect of cAMP protein kinase.

Insulin also induces synthesis of glycolytic enzymes. It signals the "fed state", stimulating both glycogen and lipid synthesis.

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Effects of Glucocorticoids

- Steroid hormones act differently by entering the cell, binding to an intracellular receptor, and regulating gene activity in the nucleus.
- Cortisol promotes protein degradation in muscle and gluconeogenesis in liver (as well as stimulation of urea cycle enzymes to complete amino acid breakdown).

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Ca^{2+} Also Stimulates Glycogen Degradation

- **Phosphorylase kinase** is activated both by phosphorylation and also by Ca^{2+}.
- It has four subunits: α, β, γ, δ
- γ is the active subunit.
- α and β are **inhibitors**. Inhibition is removed when phosphorylated by **protein kinase**.
- δ is a protein called **calmodulin**, a calcium binding protein involved in many calcium stimulated reactions.

Electrical stimulation of muscle opens calcium channels in the sarcoplasmic which stimulates muscle contraction, so glycogen breakdown can also be coordinated with the muscles need for energy.

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Pentose Phosphate Pathway
(aka Hexose Monophosphate Shunt)

- Alternative pathway for glucose oxidation.
- Major source of NADPH for biosynthetic reactions.
- Source of pentoses.
- Two categories of reactions:
  - Oxidative steps.
    - Produce NADPH and CO_2
  - Non-oxidative "shuffling" steps.
    - Interconvert hexoses and pentoses

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Oxidative Steps

- Glucose-6-P Dehydrogenase (Fig 23.27)
  - Irreversible and regulated.
- Gluconolactonase (Fig 23.28)
- 6-Phosphogluconate Dehydrogenase
  - Oxidative decarboxylation of beta-hydroxy acid. (Fig 23.29)
    - Remember isocitrate dehydrogenase and malic enzyme.

G-6-P dehydrogenase is inhibited by NADPH and by fatty acyl-CoA esters.

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Oxidative Steps, con't.

- Overall reaction:

Glucose-6-P + 2 NADP Ž ribulose-5-P + 2 NADPH + CO_2

- The only steps where oxidation occurs and CO_2 is produced.

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Non-Oxidative Steps

- Equilibrium reactions that interconvert trioses, tetroses, pentoses, hexoses and a heptose.
- Sequence of reactions depends on needs of cell. Overall stoichiometry depends on which components are pulled from equilibrium mixture.
- "Shuffling reactions" very similar to those in Calvin-Benson cycle.
  - One exception: **transaldolase** instead of **aldolase**.

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Non-Oxidative Steps, con't.

- Pentose interconversions:
  - Phosphopentose isomerase (Fig 23.30)
  - Phosphopentose epimerase (Fig 23.31)
- Transketolase (Fig 23.32)
  - Transfers two carbon unit as in C-B cycle
    - Configuration at carbon-3 of the ketose must be L.
    - Two carbon unit bound to TPP on enzyme as an intermediate. (Fig 23.34)

Notice that transketolase has a broad specificity for both the ketose donor and the aldose acceptor.

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Non-Oxidative Steps, con't.

- Transaldolase
  - Transfers a 3-carbon unit from sedoheptulose-7-P. (Fig 23.35)
  - Three carbon unit forms a Schiff base intermediate with a lysine side chain. (Fig 23.36)
  - Remember the reverse reaction in the C-B cycle was catalyzed by a special **aldolase** which produced sedoheptulose-1,7-bisphosphate.

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Variations in Stoichiometry

- Summary of overall pathway (Fig 23.26)
- Only NADPH needed (Fig 23.39)
- Only pentoses needed (Fig 23.38)
- Balance of NADPH and pentoses (Fig 23.37)
- ATP as well as NADPH needed (Fig 23.40)

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Wernicke-Korsakoff Syndrome

- Transketolase binds TPP tenfold less strongly than normal. Thiamine deficiency therefore produces a neuropsychiatric disorder that can be treated by increasing thiamine in the diet.
  - (A combination of diet and genetic condition).

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Glucose-6-phosphate dehydrogenase deficiency

- Sex linked recessive.
- Red cells need NADPH to keep **glutathione** reduced, which is needed to:
  - Keep hemoglobin in the **ferrous** state
  - Detoxify peroxides
- Causes drug induced hemolytic anemia (where drugs stimulate peroxide production).
- May provide some protection from malaria.