

Glycogen Metabolism

Glycogen is an important storage macromolecule. While we inject polysaccharides in a variety of forms, glycogen is the major carbohydrate storage form in mammals. This unit will cover the roles of glycogen, and how intracellular and extracellular signals result in glycogen synthesis or release. Finally we will discuss the consequences of aberrant glycogen storage. For more details on this topic, we recommend Bollen et al. [1998] and chapter 11 of Lippincott's Illustrated Reviews: Biochemistry¹.

¹ Denise Ferrier. *Lippincott Illustrated Reviews: Biochemistry*. LWW, 1496344499, 7th edition, 2017. ISBN 1496344499

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Learning Objectives

- Evaluate how the structure of glycogen allows for compact but accessible storage of glucose molecules.
- Understand how glycogen synthesis and glycolysis are regulated by intracellular metabolites.
- Explain how protein phosphorylation regulates Glycogen Synthase and Glycogen Phosphorylase activities and how extracellular signals affect glycogen metabolism.
- Assess the tissue-specific roles of insulin, adrenaline and glucagon in glycogen storage.
- Distinguish between the functions of glycogen in liver, adipose and muscle and evaluate how alterations in glycogen metabolism affect the physiological functions of these tissues.
- Explain how glycogen storage diseases can occur and how specific genes result in different pathophysiology depending on both the gene, and tissue where it is expressed.

Structure and function of glycogen

Glycogen is a homopolymer of glucose units connected to each other by $\alpha 1-4$ or $\alpha 1-6$ glycosidic linkages². This allows for many molecules of glucose to be compactly stored in the cell, and then made available upon energy or glucose demand. A series of $\alpha 1-4$ bonds result in a more or less straight chain of glucose molecules, while a $\alpha 1-6$ linkage results in a branch point. A single glucose moiety can have both a $\alpha 1-4$ and $\alpha 1-6$ linkage (see Figure 1).

Role of glycogen branching

The branched structure of glycogen means that a single macromolecule can be both very compact, but have many free glucose ends³. If glycogen was totally unbranched then glucose could only be released one molecule at a time from a glycogen molecule. By having many branch points, the enzymes that release glucose from glycogen⁴ can release glucose from many reducing ends at the same time. This allows for more rapid mobilization of glucose when needed.

BECAUSE BRANCH POINTS NEED TO BE REMOVED BY A SEPARATE ENZYME⁵, overly branched glycogen is extremely compact, but its digestion may be limited by how well Glycogen Phosphorylase and

² This nomenclature refers to a link between the #1 position of one molecule of glucose (the anomeric carbon) and either the #4 or the #6 position on the next glucose.

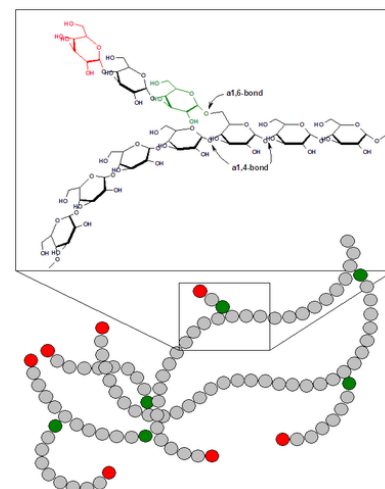


Figure 1: The structure of glycogen. Note the location of branch points ($\alpha 1-6$ glycosidic linkages in green) and reducing ends (red). From <https://commons.wikimedia.org/w/index.php?curid=611992>.

³ These ends are known as reducing ends.

⁴ known as Glycogen Phosphorylase

⁵ known as glycogen debranching enzyme or amylo- α -1,6-glucosidase (encoded by the AGL gene).

the glycogen debranching enzyme can work in concert. Defects in debranching enzyme result in a glycogen storage disease characterized by an inability to eliminate these branch points (see the section on Glycogen storage diseases below).

Different tissues use glycogen for different purposes

In principle, glycogen is an easily mobilizable source of glucose for many tissues. The content of glycogen ranges from 1-3% of total weight in muscle tissue to up to 10% of total mass in a well fed liver. Based on what we have already learned about how glucose and phosphorylation differs between cells, different tissues use glycogen for slightly different reasons. In general, muscle cells use glycogen when energy is needed, for example during exercise. Liver cells on the other hand store glycogen to make glucose available for itself and other tissues, rather than for energy.

GLYCOGEN IS AN IMPORTANT ENERGY SOURCE. Muscle tissue can have dramatic and rapid depletions of ATP in response to exercise. The initial resource to replenish ATP is the creatine phosphate system⁶ The amount of creatine phosphate can be quickly reduced, so muscle cells next turn to glycogen for glucose. These glucose molecules typically undergo glycolysis (and depending on the fiber type) then the TCA cycle and electron transport chain to make ATP⁷. As we will describe below, the primary signals for glycogen metabolism in muscle are energy dependent.

⁶ We will discuss this in the unit on non-protein compounds derived from amino acids later in the semester.

⁷ Recall, the absence of glucose-6-phosphatase means that liberated glucose-6-phosphate will not be dephosphorylated for extracellular release.

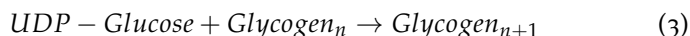
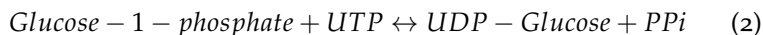
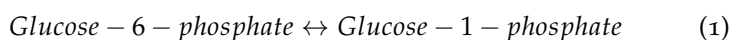
GLYCOGEN PLAYS AN IMPORTANT ROLE IN ENDURANCE EXERCISE. During prolonged exercise, muscle glycogen can be dramatically depleted. To prevent this, athletes often ingest carbohydrates during exercise in order to continuously provide energy to the contracting muscles. Another approach is to deplete glycogen levels prior to exercise, then eat a large carbohydrate rich meal. This results in *more* glycogen storage than the normal fed states, a condition known as glycogen supercompensation. For more information on this concept see Hawley et al. [1997].

GLYCOGEN FROM THE LIVER PLAYS AN IMPORTANT ROLE IN GLUCOSE HOMEOSTASIS. The liver on the other hand mobilizes glycogen in order to maintain glucose levels in blood. Whether due to fasting, or exercise the body needs to make glucose available for many tissues. This is especially important for the brain, which is very poor at converting fatty acids into ATP. As such, glycogen levels in the liver are generally controlled by indicators of glucose flux, such as

glucose-6-phosphate. Unlike the muscle, once glycogen is catabolized into glucose-6-phosphate it can be dephosphorylated and released from hepatocytes.

Regulation of glycogen synthesis

Glycogen is stored when glucose and energy are plentiful. After a typical meal, in a healthy person glycogen levels increase by about 50% peaking about 4h after a meal [Taylor et al., 1996]. This is due to a combination of increased glucose availability and the postprandial actions of insulin. Glycogen is synthesized starting from Glucose-6-phosphate via the following series of reactions:



There are two important things to note about this process, one is that the first two reversible steps mean that the levels of G6P are extremely important in this process⁸. The second is that by using UTP to activate glucose, this is an *energy consuming process*. This means that there is an energetic cost (the equivalent of one ATP phosphodiester bond) to storing glycogen. The third reaction is catalyzed by the enzyme Glycogen Synthase⁹ and that is the main point of regulatory control in glycogen synthesis.

Glycogen Synthase is activated by Glucose-6-phosphate

Both isoforms of Glycogen Synthase are allosterically activated by glucose-6-phosphate as first described in the late 1950s by Leloir et al. [1959]. G6P levels are increased when glycolysis is low, and glucose levels are high. This is generally a situation where nutrient levels are high, but energy demand is low. This is a good time to store extra glucose, so this makes physiological sense.

Extracellular control and signaling

In addition to this metabolite-level control, Glycogen Synthase is also regulated by reversible protein phosphorylation [Villar-Palasi and Lerner, 1960]¹⁰. The protein kinases that regulate Glycogen Synthase are complex, but as a general rule they result in the *inactivation* of the

⁸ Recall from the lecture on glycolysis that G6P levels are controlled by the levels of glucose in the cell, the activity of hexokinase/glucokinase and the activity of PFK1, the rate limiting step in glycolysis.

⁹ There are two isoforms of Glycogen Synthase, one expressed in muscle and brain (*GYS1*) and one expressed in the liver and fat, encoded by the *GYS2* gene.

¹⁰ This was one of the earliest described examples of metabolic control by protein phosphorylation.

enzyme. These kinases are summarized in Table 1. Since insulin *activates* Glycogen Synthase, it does this by *dephosphorylating* these sites (see Figure 2). Part of this process is by reducing the activity of the kinases (especially GSK3 and PKA) but insulin also functions by activating protein phosphatase activity towards Glycogen Synthase. This is accomplished via a series of proteins that specifically target a protein phosphatase to the glycogen particle. The precise mechanisms by which insulin promotes this dephosphorylation are still unclear. At the same time that Glycogen Synthase is being dephosphorylated (and activated), Glycogen Phosphorylase¹¹ is also dephosphorylated and inactivated.

¹¹ The enzyme that cleaves glucose from an existing glycogen molecule

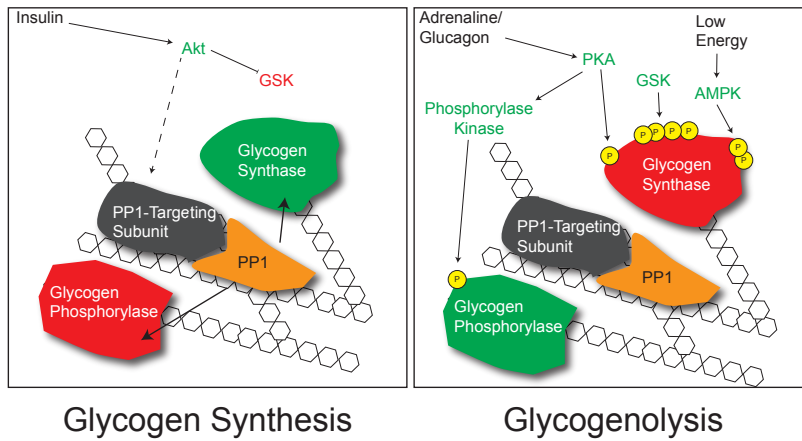
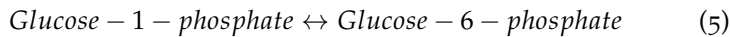
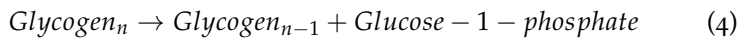


Figure 2: Post-translational regulation of glycogen metabolism. Red indicates inactive enzymes, while green indicates active enzymes. PP1 is a protein phosphatase that removes the phosphate groups from both Glycogen Synthase and Glycogen Phosphorylase. The dashed arrow indicates the mechanisms by which this phosphatase are activated are currently unknown.

SEPARATE FROM THE EFFECTS OF INSULIN ON GLYCOGEN SYNTHASE ACTIVITY, recall that insulin will also promote glucose uptake (in muscle and fat tissues). This increased glucose flux will result in more Glucose-6-phosphate in the cell and allosteric activation of Glycogen Synthase. Therefore there are at least two ways by which insulin can promote glycogenesis.

Regulation of glycogen breakdown

Glycogen is broken down to release glucose in two steps¹²:



The fate of Glucose-6-phosphate depends on the relative activities of PFK1, Glucose-6-phosphate dehydrogenase¹³ and, in the case of liver cells Glucose-6-phosphatase. Generally, in the muscle the liberated Glucose-6-phosphate enters glycolysis whereas in the liver it is dephosphorylated and released as glucose.

Table 1: Protein kinases that phosphorylate and inactivate Glycogen Synthase.

Kinase	Signal
PKA	Adrenaline/Glucagon
GSK3	Insulin (inactivates)
AMPK	Energy Stress

¹² The exception is at branch points, which will be described below

¹³ Leading towards the pentose phosphate pathway.

Intracellular control

The first, and rate limiting step of glycogenolysis is catalyzed by an enzyme named Glycogen Phosphorylase¹⁴. Glycogen Phosphorylase is allosterically activated by AMP. This activation by AMP is blocked by the presence of ATP or Glucose-6-phosphate. AMP is increased when there is energy demand, as ATP is used up, so if there is a need for energy, Glycogen Phosphorylase gets activated. This can be overridden when ATP is plentiful (indicating a lack of energy stress) or Glucose-6-phosphate is elevated (indicating glycolysis can be active). While all three isoforms respond similarly in direction, the muscle enzyme is much more sensitive to activation by AMP than the liver enzyme. This is due to structural differences in the AMP-binding pocket between the muscle and liver isoforms [Rath et al., 2000]. Glycogen Phosphorylase also uses Vitamin B₆-derived Pyridoxal phosphate as a prosthetic group¹⁵.

¹⁴ There are three isoforms of this gene, *PYGL*, *PYGM* and *PYGB* which are expressed in the liver, muscle and brain respectively.

¹⁵ Vitamin B₆ has many other roles, but has been shown to be effective in treating McArdle's disease, a genetic deficiency in *PYGM*.

Extracellular control and signaling

The activation of Glycogen Phosphorylase by AMP can be overridden by protein phosphorylation on a key residue by an enzyme named Phosphorylase Kinase. This means that once phosphorylated, the enzyme functions as if it is in the AMP-activated state. The phosphorylation of this site is activated by PKA dependent signaling, induced by either glucagon in the liver or adrenaline in liver, muscle and other tissues. Similarly, PKA-dependent phosphorylation phosphorylates and inactivates Glycogen synthase as described above. This means that adrenergic signaling turns Glycogen Phosphorylase on, and Glycogen Synthase off simultaneously¹⁶. A summary of the effects of reversible protein phosphorylation on the enzymes of glycogen metabolism is shown in Table 2

¹⁶ Also, insulin turns Glycogen Phosphorylase off, and Glycogen Synthase on.

Enzyme	Effects of Phosphorylation
Glycogen Synthase	Inactivates - Less Synthesis
Glycogen Phosphorylase	Activates - More Breakdown

Table 2: Summary of how protein phosphorylation regulates glycogen synthase and Glycogen Phosphorylase.

Regulation of glycogen branching and removal of branch points

The generation and removal of glycogen branch points is an important part of glycogen metabolism. Glycogen Phosphorylase can only cleave at α 1-4 bonds and cannot proceed past α 1-6 linkages. Whenever a α 1-6 linkage is encountered, the Glycogen Debranching Enzyme is recruited, which removes the α 1-6 link and allows for Glycogen Phosphorylase to proceed. Currently, there is no strong data

suggesting that either Glycogen Branching or Debranching Enzymes are regulated by metabolites, or hormonal signals.

Glycogen storage diseases

There are a variety of heritable defects which result in aberrant glycogen metabolism (see Table 3). Some of these result in the inability to synthesize glycogen, while others prevent glycogenolysis, resulting in pathologically large particles of glycogen resulting in cell death. Some common glycogen storage diseases, and the affected enzyme are below. Try to predict, based on what you have learned about the role of these enzymes what some potential symptoms and treatments for these diseases may be.

Disease	Enzyme	Glycogen	Phenotype
Fanconi-Bickel syndrome	<i>SLC4A2</i>		
von Gierke's disease	<i>G6PC</i>		
Tarui's disease	<i>PFKM</i>		
GSD Type o	<i>GYS2</i>		
Cori's disease	<i>AGL</i>		
Andersen disease	<i>GBE</i>		
McArdle disease	<i>PYGM</i>		
Hers' disease	<i>PYGL</i>		
GSD type IX	<i>PHKA1/2</i>		

Table 3: Some example glycogen storage diseases and the gene that is mutated. *G6PC*: Glucose-6-phosphatase; *PFKM*: Muscle PFK1; *PHKA1/2*: Phosphorylase kinase; *SLC4A2*: GLUT2.

References

Mathieu Bollen, S Keppens, and Willy Stalmans. Specific features of glycogen metabolism in the liver. *The Biochemical journal*, 336 (Pt 1:19–31, 1998. ISSN 0264-6021. DOI: 10.1042/bj3360019. URL <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1219837&tool=pmcentrez&rendertype=abstract>.

Denise Ferrier. *Lippincott Illustrated Reviews: Biochemistry*. LWW, 1496344499, 7th edition, 2017. ISBN 1496344499.

John A. Hawley, Elske J. Schabort, Timothy D. Noakes, and Steven C. Dennis. Carbohydrate-Loading and Exercise Performance. *Sports Medicine*, 24(2):73–81, aug 1997. ISSN 0112-1642. DOI: 10.2165/00007256-199724020-00001. URL <http://link.springer.com/10.2165/00007256-199724020-00001>.

L F Leloir, J M Olavarria, S H Goldemberg, and H Carminatti. Biosynthesis of glycogen from uridine diphosphate glucose. *Archives*

of biochemistry and biophysics, 81(2):508–20, 1959. ISSN 0003-9861.
URL <http://www.ncbi.nlm.nih.gov/pubmed/13638016>.

V L Rath, M Ammirati, P K LeMotte, K F Fennell, M N Mansour, D E Danley, T R Hynes, G K Schulte, D J Wasilko, and J Pandit. Activation of human liver glycogen phosphorylase by alteration of the secondary structure and packing of the catalytic core. *Molecular cell*, 6:139–148, 2000. ISSN 10972765. DOI: 10.1016/S1097-2765(05)00006-7.

R Taylor, I Magnusson, Douglas L. Rothman, G W Cline, A Caumo, C. Cobelli, and Gerald I. Shulman. Direct assessment of liver glycogen storage by ^{13}C nuclear magnetic resonance spectroscopy and regulation of glucose homeostasis after a mixed meal in normal subjects. *Journal of Clinical Investigation*, 97(1): 126–132, 1996. ISSN 0021-9738. DOI: 10.1172/JCI118379. URL <http://www.jci.org/articles/view/118379>.

Carlos Villar-Palasi and Joseph Larnier. Insulin-mediated effect on the activity of UDPG-glycogen transglucosylase of muscle. *Biochimica et Biophysica Acta*, 39(1):171–173, mar 1960. ISSN 00063002. DOI: 10.1016/0006-3002(60)90142-6. URL <http://linkinghub.elsevier.com/retrieve/pii/0006300260901426>.