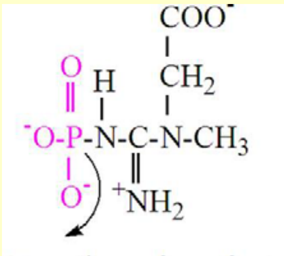
Phosphate Nutrients

The more you study cellular energy metabolism, the more you gain an appreciation for the importance of phosphate groups (HPO_3^{-2}). Many of the reactions of the phosphagen system, glycolysis and the TCA cycle move phosphate groups so that they are located in more easily accessible regions of a molecule that favors cleavage (**dephosporylation**) from one substrate for addition (**phosphorylation**) to another, to form a different product. Creatine phosphate is one example of a phosphorylated intermediate of skeletal muscle energy catabolism, where the phosphate group is transferred to the molecule ADP to form ATP in a reaction catalyzed by the enzyme creatine kinase.



Creatine phosphate

You will also learn that phosphate groups are pivotal in the process of muscle contractile failure during intense exercise. The double negative charge of HPO_3^{-2} causes charge attraction to the double positive charge of free calcium ions (Ca^{+2}). Thus, during intense muscle contractions there is an increased accumulation of intramuscular phosphate groups. Due to the charge interaction between phosphate and calcium (HPO_3^{-2} - Ca^{+2}) more and more calcium is prevented from effective re-uptake and release into and from the sarcoplasmic reticulum. This results in a progressive decline in available calcium to bind with troponin, which in turn decreases the force of muscle contraction.

Adenylates

The structure of **ATP** is presented in Figure 1. The three phosphate groups are identified by color coding. ATP consists of three functional groups; an **adenosine** group, a **ribose** sugar group, and the three phosphates connected together in series. The **standard Gibbs' free energy change (ΔG°')** for ATP hydrolysis is -7.3 Kcal/M. The **absolute delta G (ΔG)** approximates -9.7 Kcal/M at rest. Yes, far greater free energy release than the ΔG°'! However, after intense exercise to muscular fatigue, the ΔG to approximately -6.5 Kcal/M.

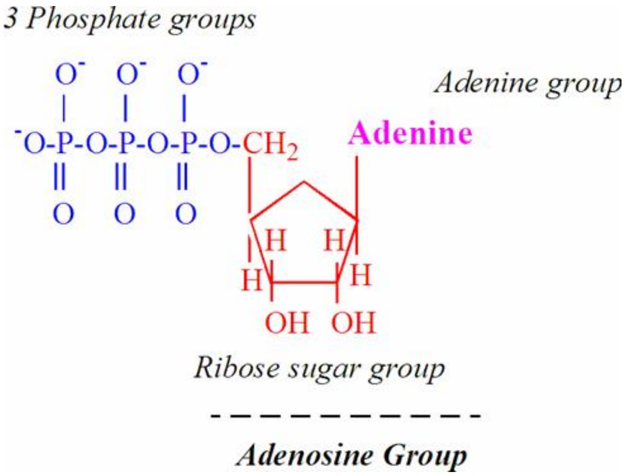


Figure 1. The chemical structure of ATP. The three phosphate groups are colored blue, the ribose sugar red and the adenine base word magenta. Adenosine is the nucleoside base comprising ribose and adenine.

I want you to develop your own Figure comparing the ΔG°' of ATP to other phosphorylated intermediates of energy metabolism. Use the data of Table 1 to do this. What do you observe about where ATP falls with regard to the free energy release of phosphorylated intermediates? If you have been observant, you would have realized that ATP has a moderate ΔG°', with **phosphoenolpyruvate** releasing the greatest amount of free energy under standard conditions (-14.8 Kcal/mol). At first impression, you might think that the larger the free energy release, the better. However, why would a higher ΔG°' for ATP hydrolysis not be suitable for a cell?

Table 1. The standard free energy release (ΔG°') data* for key phosphorylated intermediates of energy catabolism.

Phosphorylated Compound	Free Energy* Kcal/mol	Free Energy* kJ/mol
Phosphoenolpyruvate	**-14.8**	-61.9
1,3 bisphosphoglycerate	**-11.8**	-49.3
Creatine phosphate	**-10.3**	-43.0
ADP	**-7.3**	-30.5
ATP	**-7.3**	-30.5
Glucose-1-phosphate	**-5.0**	-20.9
Fructose-6-phosphate	**-3.8**	-15.9
AMP	**-3.4**	-14.2
Glucose-6-phosphate	**-3.3**	-13.8

*For the removal of 1 phosphate group, standard conditions

Think about the importance of ATP as the main free **energy currency** of a cell. The cell has to regenerate ATP, as well as use it to release free energy to fuel cell work. Thus, ATP must provide sufficient free energy during its breakdown, as well as not require too much free energy to prevent it from being formed during catabolism. As such, it is logical that ATP has a moderate free energy requirement. ATP can then be regenerated by coupling this reaction to more exergonic reactions. Conversely, the free energy of ATP hydrolysis can be used when the reaction is coupled to cell processes such as muscle contraction, molecular transport, protein synthesis, etc.

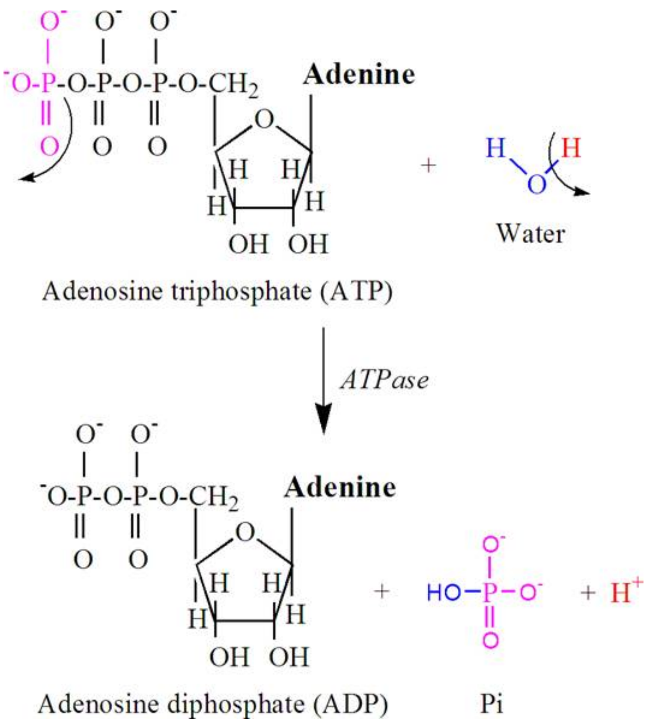


Figure 2. The substrates and products of ATP hydrolysis. Note the color identification of atoms and electrons involved in the phosphate transfer and hydrolysis of water.

I have used the term **ATP hydrolysis** several times already without adequate explanation. Figure 2 presents the ATPase reaction, illustrating the role of water in the reaction, and hence the term ATP hydrolysis. Also be aware of the water source of the proton released during this reaction, as this is important for understanding the biochemistry of proton release during intense exercise.

Is ATP An Energy Store?

I wanted to address this question in this section to be thorough in content, and also deal with this question in the beginning of your study of energy metabolism. When I was a student, not that different to you, I was taught that the muscle store of ATP was an energy store, and that one of the energy systems of muscle was the ATP-CrP system. While muscle ATP does function to transfer free energy, it is wrong to refer to it as an energy store. To do so would imply that like CrP, the muscle store of ATP can decrease to near zero. Such interpretations fostered by treating ATP as an energy store in discussion and writing is wrong based on application of the second law of thermodynamics, the role of ATP as a transfer agent of free energy, and the wealth of research evidence that clearly shows minimal decreases in muscle ATP during voluntary intense exercise to exhaustion. The greatest reduction in muscle ATP in humans during voluntary exercise involves a change from 8.2 mmol/kg wet wt to 6.5 mmol/kg wet wt; a decrease of only 21%. Also remember that such a decrease is associated with profound muscular contractile failure. In reality, decreases in muscle ATP coincide with excessive **muscular fatigue** and metabolic failure. Why is this so?

Remember the bioenergetics of ATP hydrolysis. The free energy release of ATP hydrolysis is dependent on the concentrations of substrates and products (Equation 1, Figure 2). If muscle ATP decreased, what would happen to the free energy release of ATP hydrolysis? Yes, it would decrease, meaning less energy for cells to perform essential work, which in turn would cause impaired function of essential processes of the cells. For skeletal muscle, this translates to impaired muscle contraction.

$$ATP + H_2O \overset{ATPase}{\rightarrow} ADP + Pi + H^+$$ Equation 1

Clearly, it would be devastating to the muscle cell if muscle ATP decreased too much. The physiologically valid decreases in muscle ATP from 8.2 to 6.5 mmol/kg wet wt coincides with a decrease in the absolute Gibbs' free energy change of -9.7 to -6.5 Kcal/M. Of course, this also involves an increase in muscle ADP and Pi, which can both also independently contribute to muscle fatigue. Thus, the 21% decrease in cellular ATP causes a 33% decrease in the ΔG of ATP hydrolysis. If such decreases continued, then there would be far less free energy release for ATP hydrolysis and therefore less energy for the cell to do work. It is no wonder that small decreases in cell ATP coincide with severe cellular dysfunction, which once again for skeletal muscle means contractile failure.

Hexose and Triose Phosphates

In contracting skeletal muscle, the pathway of glycolysis involves the conversion of glucose to 2 pyruvate molecules. During this conversion, glucose is initially phosphorylated to glucose-6-phosphate using ATP, isomerased to fructose-6-phosphate, and phosphorylated again at the expense of another ATP to fructose-1,6-bisphosphate. These intermediates are **hexose phosphates** (Figure 3) and can accumulate to a small degree in muscle during intense exercise.

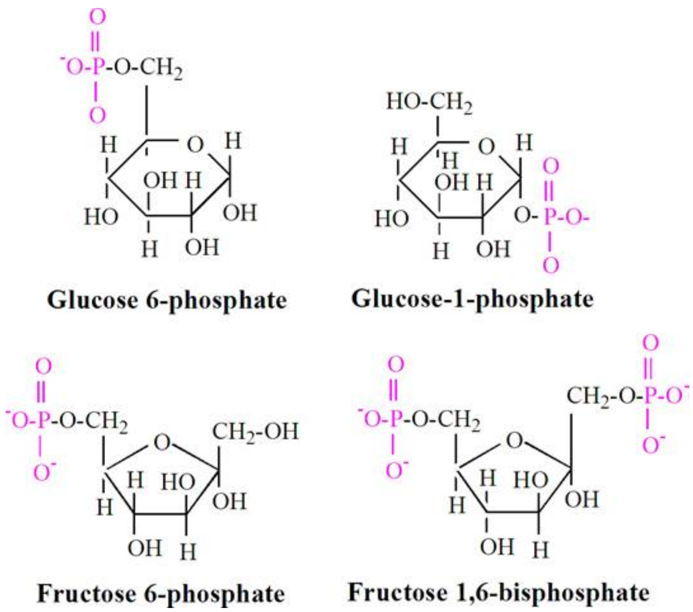


Figure 3. Examples of hexose phosphate compounds of glycolysis.

Fructose-1,6-bisphosphate is converted to 2 glyceraldehyde-3-phosphate molecules at the start of the second phase of glycolysis, and the next 5 reactions all involve 3 carbon phosphorylated compounds referred to as **triose phosphates** (Figure 4), eventually producing 2 pyruvate molecules (a 3 carbon keto carboxylic acid).

As shown in Table 1, the free energy of hydrolysis of a single phosphate from these molecules varies between the hexose and triose phosphates, with the single phosphate containing phosphoenolpyruvate having the highest standard Gibb's free energy release. In fact, as will be

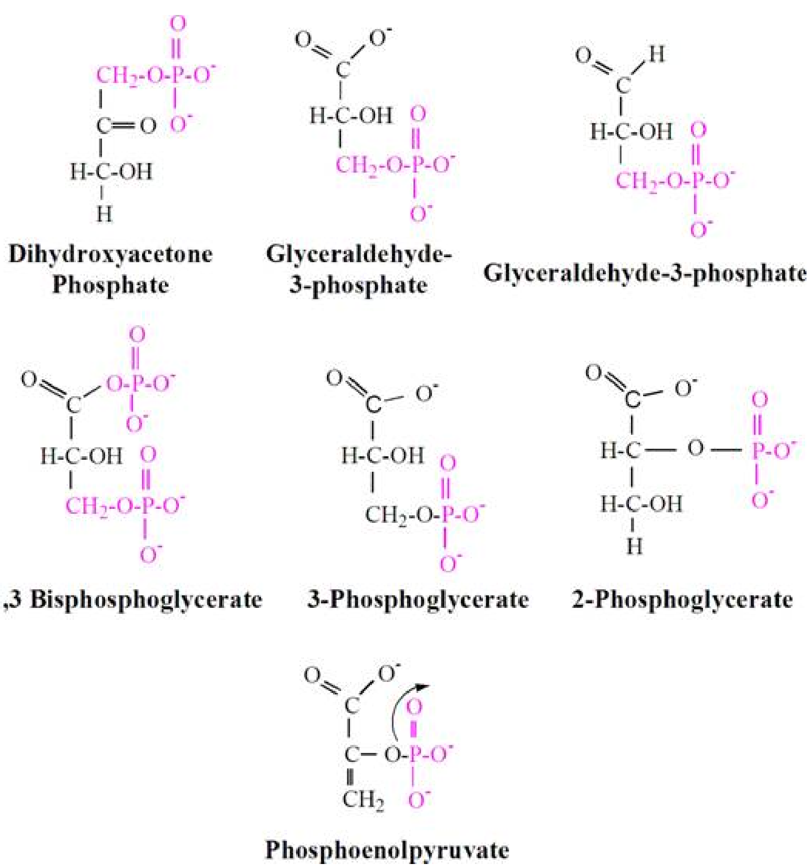


Figure 4. Examples of triose phosphate compounds of glycolysis.

learned when you study glycolysis, this is one reason why there are 9 reactions to glycolysis; to eventually modify glucose to where there is a potentially greater free energy yield from the tail end of glycolysis so that more ATP can be regenerated.

Creatine Phosphate

Creatine phosphate, **creatine** and **inorganic phosphate** (Figure 5), as well as ATP, ADP and a proton (H^+) are involved in the **creatine kinase reaction**. Creatine is produced from 2 amino acids; glycine and arginine, and is also dependent on a third amino acid, methionine, for a methyl group addition. The important role of creatine in the production of creatine phosphate has resulted in most cells of the body being able to produce creatine. However, despite the amino acid origin of creatine, the production of creatine and subsequent conversion to creatine phosphate is tightly regulated in the cell and is negligibly responsive to training or creatine supplementation in individuals with a well-balanced diet.

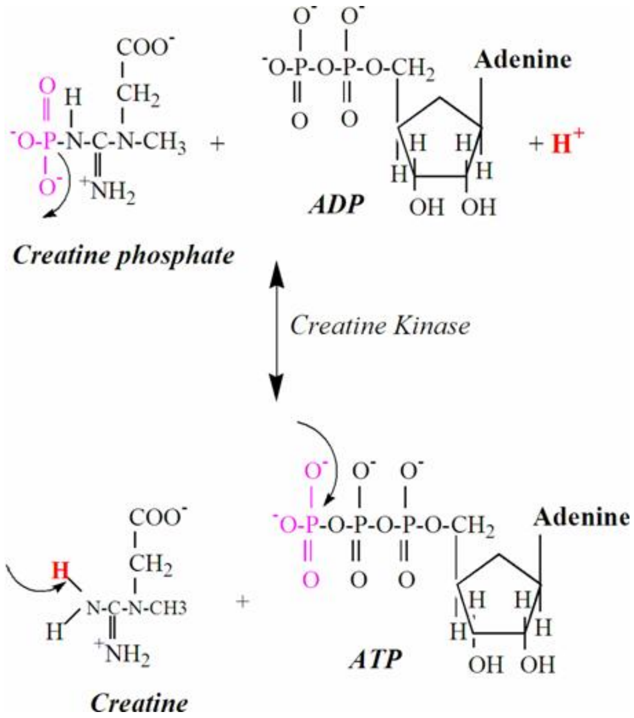


Figure 5. The chemical structures of creatine, creatine phosphate and inorganic phosphate of the creatine kinase reaction.

Glossary Words

dephosphorylation refers to the removal of a phosphate group from a molecule.
phosporylation refers to the addition of a phosphate group to another molecule.

ATP is the chemical abbreviation for adenosine triphosphate.

adenosine is a nucleotide base consisting of adenine and ribose.

standard Gibbs' free energy change (ΔG°') refers to the theoretical free energy release for a chemical reaction that commences with 1 M for each of the substrates and products at a temperature of 25 ºC and pH of 7.0.

absolute delta G (ΔG) refers to the free energy release for a chemical reaction that occurs in a cell for whatever specific conditions exist for substrates, products, temperature and pH.

Phosphate Nutrients

ribose is a 5-carbon sugar.

phosphoenolpyruvate is a 3-carbon glycolytic intermediate than is converted to pyruvate in the pyruvate kinase reaction.

energy currency is the term given to the flow of free energy via ATP in cellular metabolism.

ATP hydrolysis involves the breakdown of ATP to ADP and Pi, with the release of free energy.

muscular fatigue is defined in exercise physiology as a decreased contractile force of skeletal muscle despite sustained or continued effort.

hexose phosphates are 6-carbon sugars with phosphate group(s) attached.

triose phosphates are 3-carbon sugars with phosphate groups attached.

creatine phosphate is the main stored phosphorylated intermediate of the phosphagen system.

creatine is the nitrogenous structure forming the main chemical component of creatine phosphage.

inorganic phosphate is the common term for the HPO_3^{-2} molecule.

creatine kinase reaction is an equilibrium reaction that either transfers the phosphate from creatine phosphate to ADP, forming ATP (during increased energy demand such as muscle contractions), or uses ATP to reform creatine phosphate (during recovery).