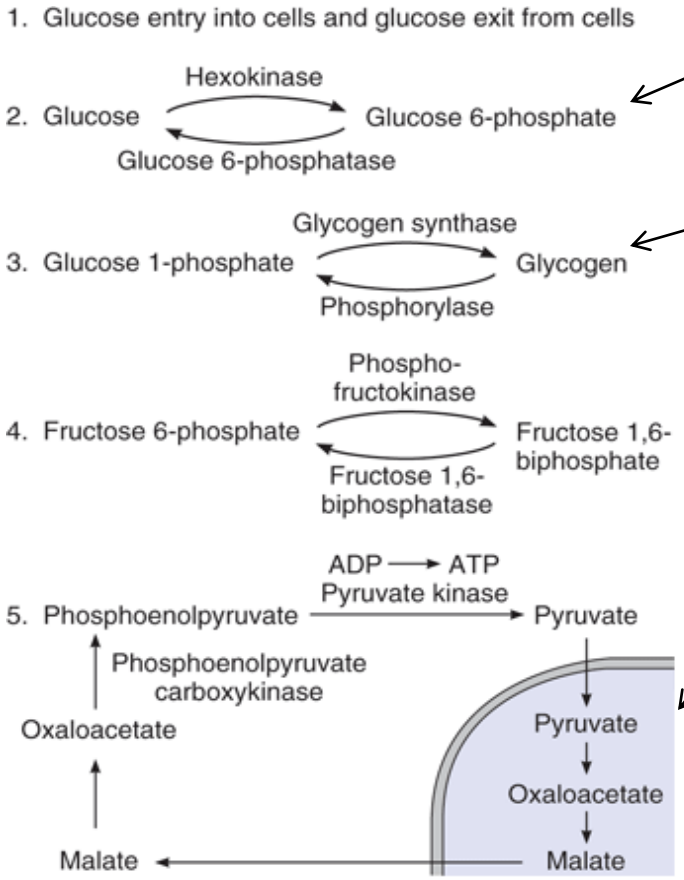
CARBOHYDRATE METABOLISM

Kenneth Alonso, MD, FACP

Directional flow



Glucokinase conversion of glucose in the liver is irreversible. Hexokinase, a glucokinase isomer, operates at a lower K_m. (muscle)

Glycogen phosphorylase B is the rate limiting enzyme.

PFK catalysis is the rate limiting step in glucose metabolism

Pyruvate carboxylase is the rate limiting enzyme in gluconeogenesis (in the mitochondrion).

Fig. 1-23 Accessed 07/01/2010

Source: Barrett KE, Barman SM, Boitano S, Brooks H: *Ganong's Review of Medical Physiology, 23rd Edition*: http://www.accessmedicine.com

Fasting state in the liver

- Glycogen is catabolized to glucose-6-phosphate.
- Pyruvate is generated via glycolysis, producing Acetyl-CoA.
- Excess glucose-6-phosphate is catabolized to glucose and enters the circulation.
- Amino acids, glycerol, lactate enter the TCA cycle, generating Acetyl-Co-A.
- Fatty acids are catabolized to Acetyl-CoA.
- The ketone bodies acetoacetate and β-hydroxybutyrate are used by muscle and brain where they are metabolized to 2 molecules of Acetyl-CoA.

Fed state in the liver

- Glucose is phosphorylated.
- Glucose-6-phosphate either enters the HMP (pentose pathway shunt)
- Produces ribose-5-phosphate for nucleotide synthesis
- Reduces NADP for fatty acid and steroid biosynthesis as well as to maintain reduced glutathione.
- OR glucose-6-phosphate undergoes glycolysis.
- Glucose in excess of energy needs is converted to and stored as glycogen.

Fed state in the liver

- Amino acids are catabolized and enter the glycolytic/ TCA cycles or are utilized for protein synthesis.
- Fatty acids enter the TCA and glycolytic cycles.
- Fatty acids in excess of energy needs are stored as cholesterol and triglycerides.

Intermediary metabolism: glycolysis

- In glycolysis, glucose is transformed to pyruvate.
- Kinases, dehydrogenases and isomerases are important enzymes in the pathway.
- Kinases transfer a phosphate atom to or from ATP.
- They consist of at least two domains, one of which binds ATP, and the other, the substrate.
- Magnesium dependent.
- Conformational changes are an important part of the catalysis by isomerases.

Intermediary metabolism: glycolysis

- Phosphorylation of glucose to glucose-6-phosphate (G6P) via glucokinase catalysis (irreversible).
- Glucokinase is only found in the liver.
- Operative only with high levles of glucose (high Km).
- Hexokinase (glucokinase isomer) is found in other organs and is feedback inhibited by G6P.
- Operative even in low levels of glucose (low Km)

Intermediary metabolism: glycolysis

- Phosphorylation of fructose-6-phosphate to fructose-1,6-biphosphate (F1,6-bisP) via phosphofructokinase 1 (PFK 1) catalysis (irreversible).
- Limiting step in glucose metabolism.
- Simulated by AMP; inhibited by ATP.
- However, they are not important for its regulation.

Intermediary metabolism: glycolysis

- Insulin stimulates PFK 2, driving production of fructose-2,6- biphosphate, stimulating PFK 1 (positive effector).
- AMP releases ATP inhibition. (ATP):(AMP) = approx. 50, while (ATP):(ADP) = approx. 10.
- Thus (AMP) changes more and is much more sensitive measure of (ATP) change and thus availability.
- In the second half energy is yielded by ATP and NADH formation.
- A total of 2 ATP and 2 NADH are gained in the glycolysis pathway.

Intermediary metabolism: glycolysis

- Glucose has now been rearranged into a molecule that can be cleaved to two interconvertible trioses.
- Energy has been consumed in the reaction.

Intermediary metabolism: glycolysis

- Oxidative phosphorylation of F-1,6-bisP to glyceraldehyde-3-phosphate (3PGA) and dihydroxyacetone phosphate (DHAP).
- DHAP is the principal product.
- It is converted to an enediol intermediate by a high activity enzyme, triosephosphate isomerase (TPI), and continues to drive the glycolytic pathway.
- DHAP enters the mitochondrion via the glycerol phosphate shuttle.

Intermediary metabolism: glycolysis

- GA-3-P is oxidized to 1,3-bisPGA via a thiol intermediate and then to 3PGA with subsequent transfer of the phosphate to ATP.
- Energy expended is regained at this point.

Intermediary metabolism: glycolysis

- Bisphosphoglycerate mutase (BPG) catalyses the interconversion of 3PGA to 2-PGA.
- The low energy compound 2-PGA is converted to the high energy compound phosphoenolpyruvate (PEP) by an alcohol elimination reaction with very little change in energy overall.
- PEP then spontaneously tautomerizes to pyruvate via pyruvate kinase catalysis (irreversible).
- Product inhibition by pyruvate and MgATP; F-1,6-bisP is a positive effector for the reaction. Glucagon promotes reaction.

Intermediary metabolism: glycolysis

- 12 ATP equivalents produced per Acetyl-CoA.
- The resulting pyruvate may now go on to the TCA Cycle or be converted into lactate.
- NAD regenerated.

Intermediary metabolism: gluconeogenesis

- Gluconeogenesis is the formation of carbohydrate from non-carbohydrate precursors.
- Three irreversible steps in the glycolytic pathway cannot be used in gluconeogenisis in the cell
- These are bypassed by reactions catalyzed by non-glycolytic enzymes.

Intermediary metabolism: gluconeogenesis

- Pyruvate is dehydrogenated to Acetyl-CoA in mitochondria via pyruvate dehydrogenase catalysis (irreversible).
- Requires vitamins B1 (thiamine pyrophosphate), B2 (riboflavin), B3 (niacin), B5 (pantohenate), and lipoic acid. Regenerates NAD.
- Lack of vitamin B1 may back-up pyruvate cycle, leading to lactic acidosis.

Intermediary metabolism: gluconeogenesis

- In the mitochondria, pyruvate carboxylase adds bicarbonate to pyruvate with the expenditure of one ATP equivalent of energy.
- Biotin, a carboxyl-group transfer cofactor, is required by this enzyme.
- This step is activated by Acetyl-CoA.
- Pyruvate carboxylase is the rate limiting enzyme.
- Oxaloacetate is decarboxylated with a simultaneous phosphorylation by GTP.
- PEP is generated by PEP carboxykinase.
- (OAA must be converted into malate or aspartate in order to cross the mitochondrial membrane.)

Intermediary metabolism: gluconeogenesis

- F1,6-bisP is catabolized by its biphosphatase. Cortisol stimulates the reaction.
- G6P is generated.
- G6P is then catabolized by its phosphatase to glucose.
- This bypasses the glucokinase reaction.
- G6P is found only in liver and kidney.
- 6 ATP equivalents needed to generate glucose from pyruvate.
- Gluconeogenesis and glycolysis are reciprocally regulated.

Intermediary metabolism: gluconeogenesis

- Glycolysis is stimulated and gluconeogenesis is inhibited by high levels of AMP and F-2,6-bisP.
- Citrate, stimulates gluconeogenesis and inhibits glycolysis.
- Another key control is the allosteric regulation of pyruvate kinase.

Anerobic glycolysis

- Anerobic glycolysis permits muscle to function in time of stress.
- In the absence of Oxygen, hydrogenated Coenzyme Q cannot be re-oxidized in the mitochondrion.
- ATP cannot be generated in the mitochondrion.
- β-oxidation and the malate shuttle are halted as are NAD+ dependent.
- NADH increases, blocking the TCA cycle.
- Pyruvate cannot be converted to the high energy compound PEP, but is rather metabolized to lactate, generating only 2 ATP.

Anerobic glycolysis (Cori cycle)

- Lactate is carried via the circulation to the liver where it is converted to pyruvate via lactate dehydrogenase.
- Metabolic burden shifted from muscle to liver at a net loss of 4 ATP per gluconeogenesis cycle.
- Alanine and glutamine are carriers of amino groups from muscle to liver (alanine is converted to pyruvate).
- Erythrocytes metabolize glucose anerobically and depend solely on glycolysis.
- Glucose is converted to pyruvate and then lactate.

Tricarboxylic acid cycle (Krebs cycle, citric acid cycle)

- As a link between the glycolysis pathway and the citric acid cycle, acetyl CoA is formed from pyruvate by the pyruvate dehydrogenase complex in the mitochondrion.
- ATP, NADH, acetyl CoA inhibit generation of acetyl CoA from pyruvate.
- Acetyl CoA is then combined with oxaloacetate to form citrate by citrate synthase.
- Citrate is how Acetyl CoA is transported.

Tricarboxylic acid cycle (Krebs cycle, citric acid cycle)

- Citrate is oxidized.
- Isocitrate dehydrogenase is the rate limiting step.
- Isocitrate is transformed irreversibly to an α-ketoglutarate by the keoglutarate dehydrogenase complex.
- Requires vitamins B1 (thiamine pyrophosphate), B2 (riboflavin), B3 (niacin), B5 (pantothenate), and lipoic acid.
- NAD is regenerated.
- ATP inhibits the reaction.

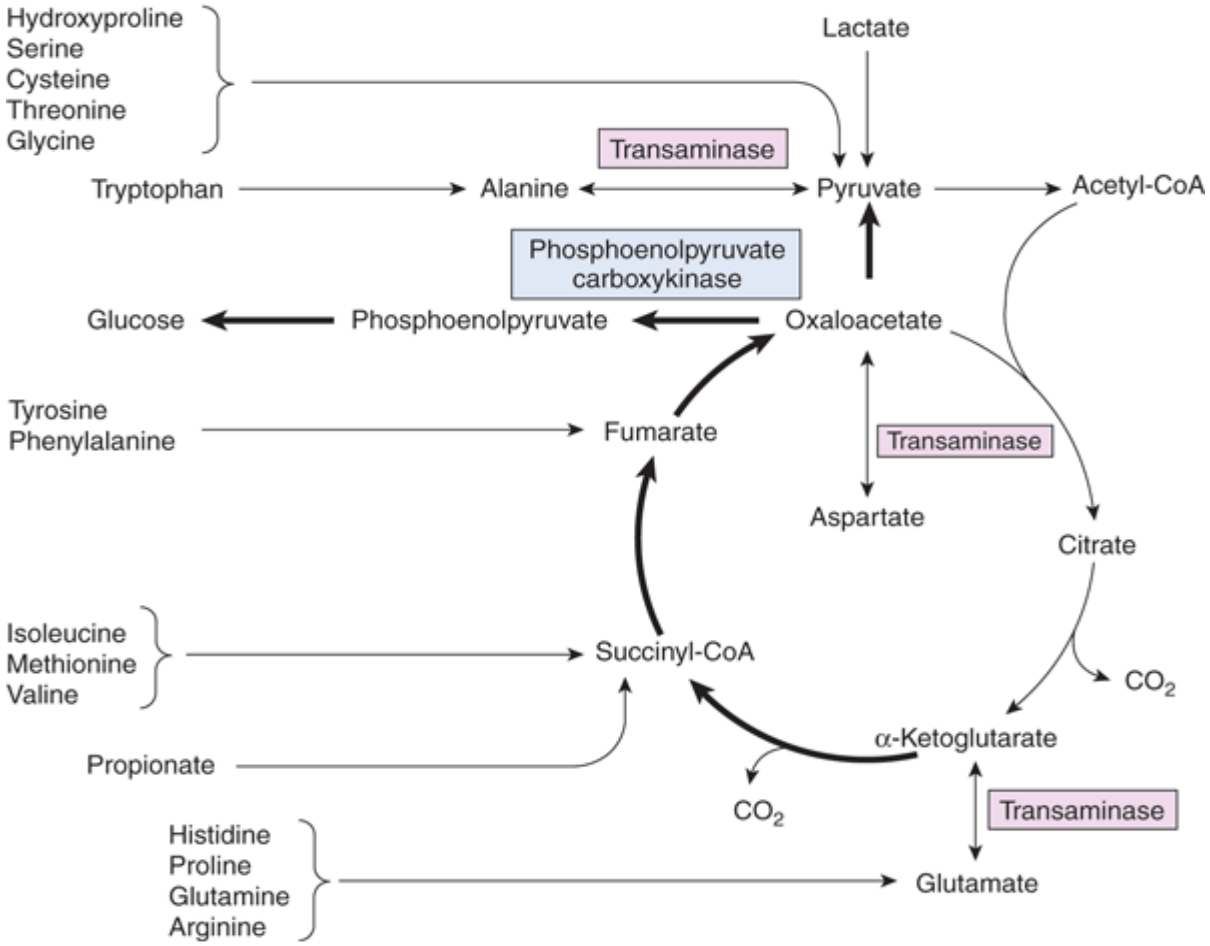
Tricarboxylic acid cycle (Krebs cycle, citric acid cycle)

- Alpha-ketoglutarate is metabolized to succinate.
- Energy is captured using Succinyl-CoA Synthase, to give a GTP which is energetically equivalent to an ATP.
- Succinate dehydrogenase, an inner-mitochondrial membrane-bound enzyme and member of the mitochondrial electron transport system (ETS), oxidizes succinate to fumerate, and, fumerase, via a hydration reaction, to malate.
- NADH and succinyl CoA inhibit the conversion.

Tricarboxylic acid cycle (Krebs cycle, citric acid cycle)

- Malate Dehydrogenase catalyzes the dehydrogenation of malate to regenerate the original carrier, oxaloacetate, for a new cycle.
- For the entire cycle we then have the production of 10 ATP/acetyl-CoA or 20 ATP/Glucose (32 ATP max).

Tricarboxylic acid cycle (Krebs cycle, citric acid cycle)



Source: Barrett KE, Barman SM, Boitano S, Brooks H: *Ganong's Review of Medical Physiology, 23rd Edition*: http://www.accessmedicine.com

Fig. 1-19 Accessed 07/01/2010

(Reproduced with permission from Murray RK et al: *Harper's Biochemistry,* 26th ed. McGraw-Hill, 2003.)

Gluconeogenic amino acids

- Threonine to glycine to alanine, serine, cysteine to pyruvate.
- Tryptophan to alanine, serine, cysteine to pyruvate.
- Pyruvate to acetyl CoA and enter TCA cycle or pyruvate to oxaloacetate and enter the TCA cycle.
- In muscle, gut, kidney, malate to pyruvate.
- Asparagine and aspartate to oxaloacetate and enter the TCA cycle.

Gluconeogenic amino acids

- Aspartate, tyrosine, phenylalanine to fumarate and enter TCA cycle.
- Arginine, histidine, proline, glutamine to glutamate to a-ketoglutarate) and enter TCA cycle.
- Valine, isoleucine, threonine, methionine to propionyl CoA to methylmalonyl CoA to succinyl CoA and enter TCA cycle.

Phases of metabolism producing ATP

- Oxidation of fuels
- Glucose to pyruvate (generating NADH and ATP)
- Pyruvate to acetyl CoA (generating NADH)
- Fatty acids to acetyl CoA (generating NADH and $FADH_2$).
- Amino acids to TCA (Krebs, citric acid cycle). (generating NADH and $FADH_2$). Nitrogen to urea cycle.
- Generation through oxidative phosphorylation. Use of NADH and $FADH_2$.

Fructose pathways

- Fructose converted by fructokinase (rapidly) with ATP to fructose-1-phosphate in liver, kidney, intestine.
- With aldolase (B in muscle or A in liver), fructose-1-phosphate converted to dihydroxyacetone phosphate
- OR is converted to glyceraldehyde and phosphorylated through triose kinase and enters the glycolytic pathway.
- Triose phosphate isomerase converts dihydroxyacetone phosphate to glyceraldehyde phosphate.

Fructose pathways

- Fructose conversion to fructose-6-phosphate (muscle, adipose tissue) is slow, requires hexokinase, and is inhibited by elevated glucose levels.

Other sugars

- Trehalose by trehalase to glucose
- Lactose by lactase to glucose and galactose
- Sucrose by sucrase to glucose and fructose
- Glycogen by a-amylase, hydrolysis to glucose
- Mannose by hexokinase and phosphomannose isomerase to fructose-6- phosphate
- Galactose to UDP-galactose and to glucose-1-phosphate

Pentose phosphate shunt (HMP)

- The pentose phosphate pathway generates NADPH for reductive biosynthesis and ribose 5-phosphate for the synthesis of RNA, DNA and coenzymes.
- G6P is converted to ribulose-5-phosphate.
- Transaldolase and transketolase (requiring TPP) create a reversible link between the pentose phosphate pathway and glycolysis (producing fructose-6-phosphate and 3-phospho-glyceraldehyde).
- G6PD is the rate limiting enzyme.

Pentose phosphate shunt (HMP)

- 12 NADPH can be generated for each G6P that is completely oxidized to CO_2.
- This shunt is found in muscle and adipose tissue as well.
- Only source of NADPH for red cells.

Polyol pathway

- Glucose to sorbitol through aldose reductase (and NADPH to NADP).
- Sorbitol to fructose through sorbitol dehydrogenase (and NAD to NADH).
- In diabetes when glucose (and galactose) levels are elevated, this reaction is pushed forward in the eye.
- The conversation to fructose is slow and the increased concentration of sorbitol and galactitol leads to increased intraocular pressure.

Glycogen metabolism

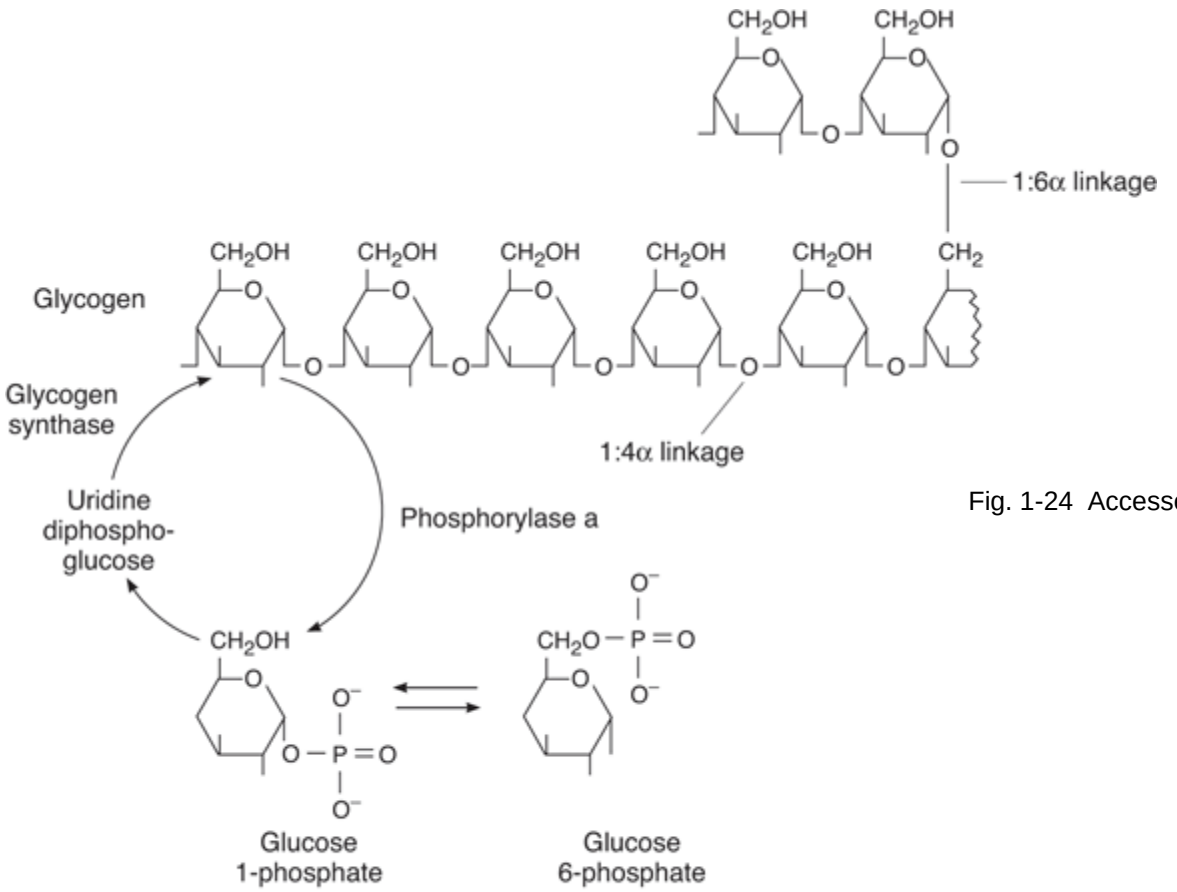


Fig. 1-24 Accessed 07/01/2010

Intermediary metabolism: glycogen formation

- Glycogen synthase covalent modification controlled by insulin and glucagon
- Insulin induces dephosphorylation.
- Dephosphorylation activates through protein phosphatase.
- Glucagon induces phosphorylation.
- Phosphorylation deactivates.
- Adenylate cyclase through protein kinase A.

Intermediary metabolism: glycogen formation

- Protein kinase A also activates phosphorylase b to a and glycogen to glucose-1-phosphate.
- In the well-fed state, glycogen will be synthesized from UDP-glucose units and stored in the liver and muscle.

Intermediary metabolism: glycogen formation

- Glycogen is a storage form of glucose that is readily accessible.
- Glycogen phosphorylase B catalyses the phosphorolytic cleavage of glycogen α-1,4 bonds to form glucose-1-phosphate.
- Free glucose only forms at branch points
- Phosphoglucomutase and glucose 1,6 biphosphate are necessary cofactors to produce G6P to permit glucose to enter the metabolic cycle.

Intermediary metabolism: glycogen formation

- Glycogen phosphorylase B contains a pyridoxal phosphate; it is stimulated by AMP, epinephrine and glucagon.
- Glycogen phosphorylase B is the rate limiting enzyme.
- Insulin stimulates glycogen synthesis (from G6P).
- This pathway is also found in muscle and adipose tissue.

Intermediary metabolism: glycogen formation

- Glycogenin is both a primer and and enzyme for the beginning of glycogen synthesis.
- Glycogenin builds a primer of glycogen on itself and then glycogen synthase takes over.
- 4:6 transferase branching enzyme
- As the glycogen molecules grows, branching enzyme moves segments.
- Branching occurs after 11 segments joined.

Energy generation

- When pyruvate is formed anerobically from glycogen, there is a net production of 3 mol of ATP per mole of glucose 6-phosphate.
- However, when pyruvate is formed anerobically from 1 mol of blood glucose, the net gain is only 2 mol of ATP.
- During aerobic glycolysis, the net production of ATP is 19 times greater than the two ATPs formed under anaerobic conditions.

Energy generation

- The conversion of pyruvate to acetyl-CoA and each turn of the TCA cycle provides four NADH and one $FADH_2$ for oxidation via the flavoprotein-cytochrome chain plus formation of one GTP that is readily converted to ATP.
- Six ATPs are formed by oxidation via the flavoprotein–cytochrome chain of the two NADHs produced when 2 mol of phosphoglyceraldehyde is converted to phosphoglycerate
- Six ATPs are formed from the two NADHs produced when 2 mol of pyruvate is converted to acetyl-CoA.

Energy generation

- 24 ATPs are formed during the subsequent two turns of the TCA cycle.
- Of these, 18 are formed by oxidation of six NADHs, 4 by oxidation of two $FADH_2$s, and 2 by oxidation at the substrate level when succinyl-CoA is converted to succinate.
- This reaction actually produces GTP, but the GTP is converted to ATP.

Energy generation

- A supply of NAD^+ is necessary for the conversion of phosphoglyceraldehyde to phosphoglycerate.
- With anaerobic glycolysis, a block of glycolysis at the phosphoglyceraldehyde conversion step might be expected to develop as soon as the available NAD^+ is converted to NADH.
- However, pyruvate can accept hydrogen from NADH, forming NAD^+ and lactate.

Energy generation

- Oxidation via the hexose monophosphate shunt generates large amounts of NADPH.
- The pentoses formed in the process are building blocks for nucleotides.
- The amount of ATP generated depends on the amount of NADPH converted to NADH and then oxidized.