GLUCONEOGENESIS

Gluconeogenesis is the formation of glucose from non-carbohydrate sources such as amino acids, lactate or lipids. Most tissues can derive energy to carry out cellular processes from glucose as well as lipids, proteins, etc. This is not the case, however, with all cells/tissues. Glucose is the **main** source of energy for the brain and skeletal muscle cells and is the **primary** energy source for erythrocytes. Therefore, this metabolic pathway is of vital importance. For example, glucose from dietary sources and from glycogen storage is depleted after approximately 18 hours of fasting. Thus, gluconeogenesis will be necessary to maintain the function of these vital cells/tissues until dietary intake is resumed or some other form of treatment is initiated. It should be noted that, during starvation, the brain, as well as cardiac muscle, can derive energy from ketones (discussed later in this lecture). Skeletal muscle can also utilize ketones to produce energy.

Approximately 90% of gluconeogenesis occurs in the liver and approximately 10% occurs in the kidney. The glucose that results from gluconeogenesis typically enters the blood stream and is distributed to the cells/tissues in need with those that utilize only glucose as first priority.

You will recall there are three steps in glycolysis that are considered to be essentially irreversible. These three steps are catalyzed by pyruvate kinase, phosphofructokinase, and hexokinase/ glucokinase. Gluconeogenesis is not an exact reversal of these steps, but rather a process sharing certain steps with glycolysis and bypassing others. The gluconeogenic pathway will require four specific enzymes to bypass these three irreversible glycolytic enzymes. In the liver, kidney cortex, and in some cases smooth muscle, the glucose-6-phosphate produced by gluconeogenesis can be incorporated into glycogen. Since skeletal muscle lacks the enzyme that bypasses the hexokinase/glucokinase reaction it cannot deliver free glucose to the blood. Therefore, in skeletal muscle, gluconeogenesis is exclusively used to generate glucose for storage as glycogen.

BYPASSING PYRUVATE KINASE

Two specialized enzymes are required to bypass *pyruvate kinase*. The first enzyme is *pyruvate carboxylase*, a mitochondrial enzyme. As the name of this enzyme implies, pyruvate is carboxylated to form OAA. The CO_2 in this reaction comes from bicarbonate (HCO_3^-). Pyruvate carboxylase requires the presence of biotin as a coenzyme. Biotin is a vitamin in the B-family. Acetyl-CoA serves as an activator for this enzyme. Acetyl-CoA is not directly involved in the reaction. The extent of accumulation of Acetyl-CoA does influence the rate of conversion of pyruvate to OAA. The following situation describes why Acetyl-CoA would be elevated.

> There is a need for energy by cells/tissues that require specifically glucose. This leads to a hormonal stimulus that increases fatty acid metabolism. Fatty acid metabolism ends with the formation of Acetyl-CoA which passes through the citric acid cycle and creates a high energy charge in this particular cell that can utilize fatty acids for energy. Since no more energy is needed by this cell, Acetyl-CoA accumulates and begins to activate pyruvate carboxylase which initiates gluconeogenesis.

The process of gluconeogenesis is illustrated on the next page.

GLUCONEOGENESIS

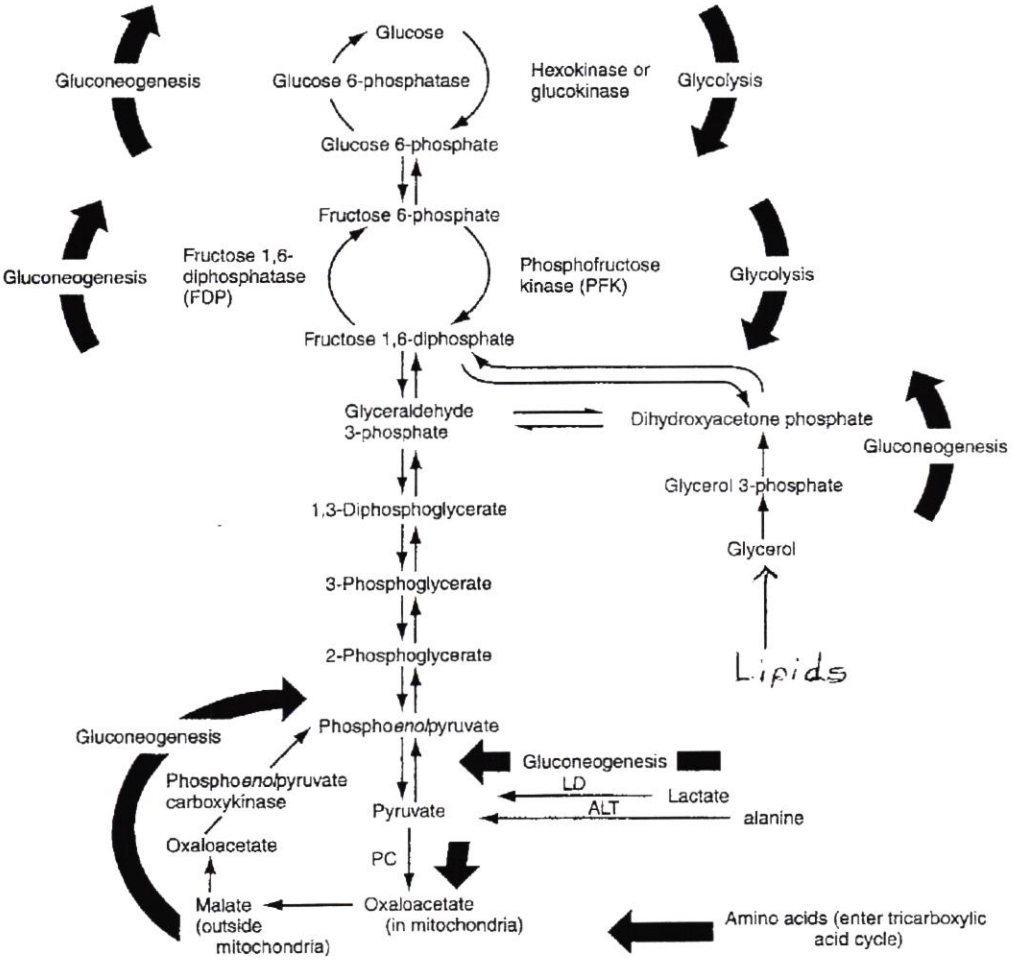


Fig. 32-6 Pathways involved in gluconeogenesis from amino acids, fatty acids, glycerol, and lactate. This pathway shares many of the enzymes of glycolytic and tricarboxylic acid pathways. Gluconeogenesis provides glucose whenever scarcity of glucose occurs and whenever lactate accumulates. *ALT,* Alanine transaminase; *LD,* lactate dehydrogenase; *PC,* pyruvate carboxylase.

For gluconeogenesis to proceed, OAA needs to be transported to the cytoplasm. However, no transport mechanism exist for this transfer and OAA will not freely diffuse into the cytoplasm. In order to enter the cytoplasm, OAA is reduced to malate in a reversal of the citric acid cycle reaction catalyzed by malate dehydrogenase. This reaction requires NADH which will be accumulating in the mitochondrion as the energy charge increases and will therefore be readily available. The resultant malate is transported to the cytoplasm where it is oxidized by cytoplasmic malate dehydrogenase and NAD to produce OAA.

The second specialized enzyme necessary to bypass pyruvate kinase is *phosphoenolpyruvate carboxykinase*. As can be seen in the previous diagram, this enzyme catalyzes the phosphorylation of OAA with GTP and a simultaneous decarboxylation. The result is phosphoenolpyruvate.

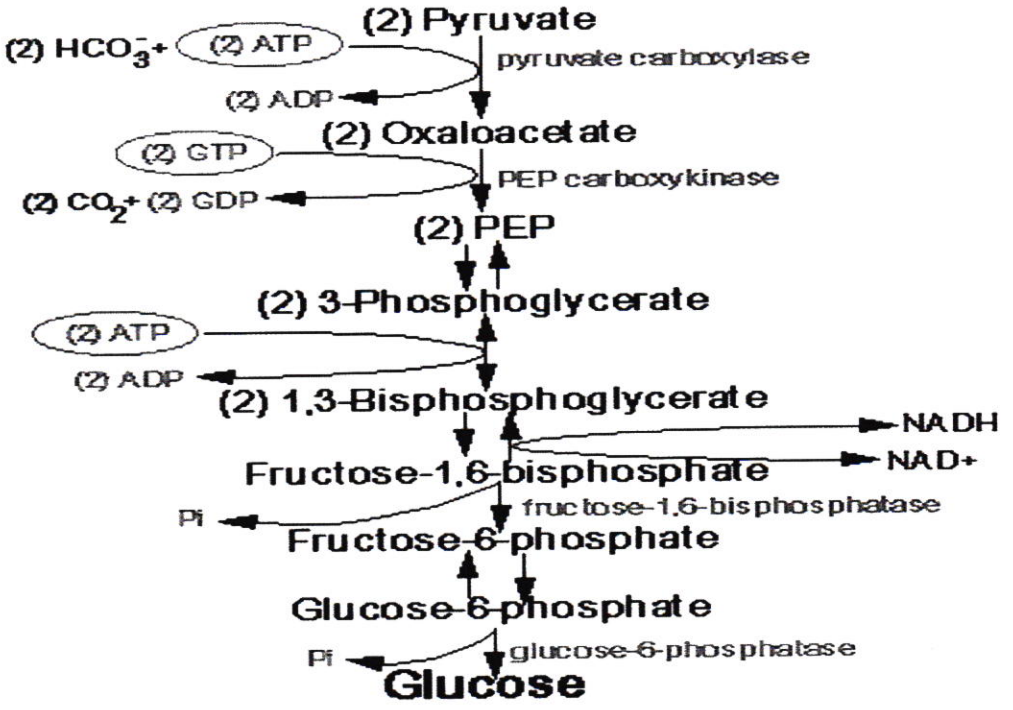
BYPASSING PHOSPHOFRUCTOKINASE

The buildup of *phosphoenolpyruvate* will shift equilibria of the reactions in glycolysis causing them to proceed in the opposite direction until the next irreversible enzyme is encountered - phosphofructokinase. The enzyme *fructose-1,6-diphosphatase* will catalyze the hydrolysis of fructose-1,6-diphosphate forming fructose-6-phosphate. Recall that the phosphofructokinase step is the rate-limiting step in glycolysis. Likewise, the fructose-1,6-diphosphatase reaction is a major control point of gluconeogenesis.

BYPASSING HEXOKINASE/GLUCOKINASE

The final irreversible reaction to be bypassed is the one catalyzed by *hexokinase/glucokinase.* The enzyme responsible for this is *glucose-6-phosphatase*. This enzyme will catalyze the hydrolysis of glucose-6-phosphate to glucose. As a result of this reaction, glucose will now be free to diffuse out of the cell, enter the blood stream and be distributed to cells/tissues that require glucose.

SUMMARY OF GLUCONEOGENESIS



In the previous diagram, the four enzymes in green are the specialized enzymes of gluconeogenesis. As can be seen, gluconeogenesis occurs at a great energy expense to the cell producing the glucose. Note that if the energy is needed immediately G6P will be the final product of gluconeogenesis and will proceed with glycolysis, if not the reaction will continue will glucose being the final product so that it may diffuse out of the cell to be delivered to those cells needing it.

REGULATION OF GLUCONEOGENESIS

Considering the pathways discussed thus far, the body has to have a mechanism to control the flow of metabolism. If these mechanisms were not in place, the body would be very inefficient by allowing reactions to proceed forward, then reverse, proceed forward, etc. There are key steps in each of the pathways considered thus far that **ensure** metabolism proceeds in one direction or the other, but **never** two different directions at one time. Consider the following sequence of events.

1. Certain cells/tissues that can **only** utilize **glucose** for energy production are in need of energy.
2. A hormonal stimulus is released to mobilize fatty acids for energy production in cells that have the ability to use fatty acids for energy.
3. Fatty acids are converted to acetyl-CoA and pass through the citric acid cycle to produce energy (ATP) in the cell stimulated by the hormone.
4. As the energy charge within the cell increases, the citric acid cycle slows.
5. With the slowing of the citric acid cycle there is a build up of acetyl-CoA which activates the enzyme pyruvate carboxylase and initiates gluconeogenesis.
6. The high levels of ATP in the cell will inhibit phosphofructokinase (the rate limiting enzyme in glycolysis), inhibit isocitrate dehydrogenase (the rate limiting enzyme in the citric acid cycle), and activate fructose-1,6-diphosphatase.
7. Inhibition of isocitrate dehydrogenase leads to a build up of citrate which will begin to be used to synthesize fatty acids. Fatty acid synthesis further inhibits phosphofructokinase.
8. Inhibition of phosphofructokinase will lead to the accumulation of glucose-6-phosphate which will inhibit hexokinase and activate glucose-6-phosphatase.
9. Glucose produced will diffuse out of the cell, enter the circulation, and be distributed to the cells in need of glucose to produce energy.
10. As can be seen in the summary of gluconeogenic reactions above, the production of glucose is at the expense of cellular energy.
11. As the need for glucose is satisfied, the hormonal stimulus is decreased, fatty acid metabolism is decreased, and acetyl-CoA production decreases.
12. The decreased level of acetyl-CoA will no longer stimulate pyruvate carboxylase to activate gluconeogenesis.
13. Recalling that ATP is used during gluconeogenesis, the lower levels of ATP will activate phosphofructokinase and inhibit fructose-1,6-diphosphatase resulting in glycolysis.
14. Activating phosphofructokinase will decrease the levels of glucose-6-phosphate which results in stimulating hexokinase and inhibiting glucose-6-phosphatase.
15. The decreased level of ATP will also activate isocitrate dehydrogenase causing the citric acid to cycle again leading to further energy production.

In addition to hormonal stimuli coming from individual cells/tissues that need specifically glucose for energy production, there are glucoreceptors located through out the body that monitor blood glucose levels. When blood glucose levels fall too low, the hormone glucagon may be released to increase blood glucose levels. The two mechanisms that respond to the glucagon stimulus are the gluconeogenic mechanism described above and glycogenolysis described below.

GLYCOGEN METABOLISM

INTRODUCTION

Stores of readily available glucose to supply the tissues with an oxidizable energy source are found principally in the liver, as glycogen. A second major source of stored glucose is the glycogen of skeletal muscle. However, skeletal muscle glycogen is not generally available to other tissues, because skeletal muscle lacks the enzyme glucose-6-phosphatase.

The major site of daily glucose consumption (75%) is the brain via aerobic pathways. Most of the remainder of glucose is utilized by erythrocytes and skeletal muscle. The body obtains glucose either directly from the diet or from lactate via gluconeogenesis. Glucose obtained from these two primary sources either remains soluble in body fluids or is stored in a polymeric form known as glycogen. Glycogen is considered the principal storage form of glucose and is found mainly in liver and skeletal muscle, with kidney and intestines adding minor storage sites. With up to 10% of its weight as glycogen, the liver has the highest specific content of any body tissue. Skeletal muscle has a much lower amount of glycogen per unit mass of tissue, but since the total mass of muscle is so much greater than that of liver, total glycogen stored in muscle is about twice that of liver. Stores of glycogen in the liver are considered the main buffer of blood glucose levels.

Recall that prior to glycolysis, glucose was trapped in the cell as glucose-6-phosphate by the following reaction. From the point of glucose-6-phosphate, metabolism may go in several directions depending on the needs of the body. If the body needs immediate energy G6P will proceed through the glycolytic pathway, but under the appropriate conditions, glucose-6-phosphate will proceed through glycogen synthesis instead.

GLYCOGEN SYNTHESIS

GLYCOGENESIS - The formation of glycogen.

The first series of steps in the synthesis of glycogen involve the formation of uridine diphosphate glucose (UDP-glucose or UDPG). The significance of this is UDP-glucose will serve as the source of glucosyl residues (i.e., glucose subunits) used to build glycogen. Glycogen can be thought of as a glucose polymer and typically glucosyl residues are added to pre-existing glycogen molecules. The first step in this series involves the conversion of glucose-6-phosphate to glucose-1-phosphate by the enzyme phosphoglucomutase. Recall that mutase enzymes typically catalyze the transfer of phosphate groups within a molecule.

Glucose-1-phosphate is next converted to UDPG by the enzyme UDPG pyrophosphorylase. This reaction requires the availability of uridine triphosphate (UTP) which is an energetic equivalent of ATP. This enzyme catalyzes the attack of the phosphate group of glucose-1-phosphate on the innermost phosphate group of UTP. The result of this reaction is UDPG and pyrophosphate (PP_i). Pyrophosphate is converted to inorganic phosphate by a side reaction. This reaction serves to activate glucose so it can be used by the next enzyme in the sequence for the formation of glycogen.

UDPG is next acted on by the enzyme glycogen synthase. This enzyme utilizes UDPG as one substrate and the non-reducing end of glycogen as another. The energy of the phospho-glucosyl bond of UDPG is utilized by glycogen synthase to catalyze the incorporation of glucose into glycogen. UDP is subsequently released from the enzyme as.

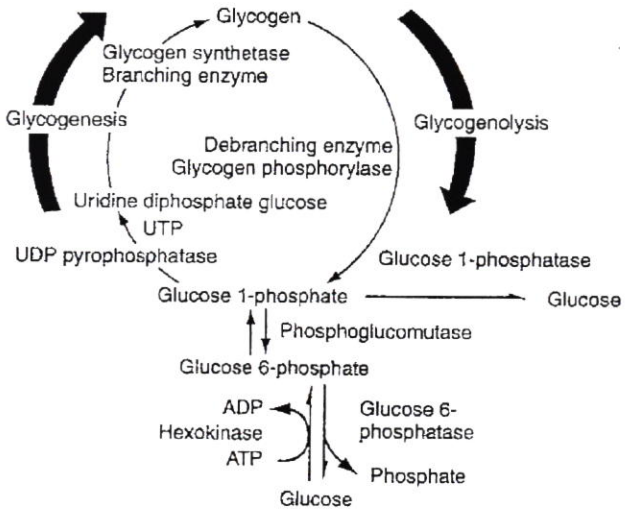
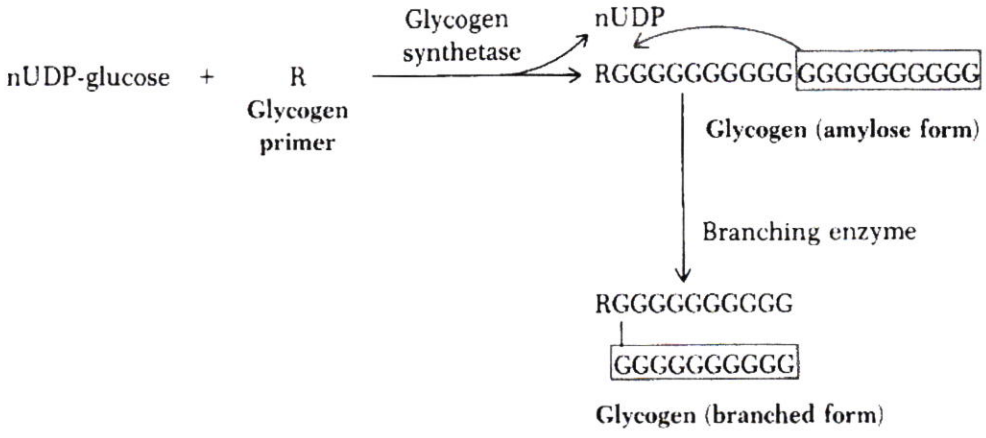


Fig. 32-5 Glycogen, storage molecule for glucose, is synthesized from glucose-1-phosphate by a process called *glycogenesis (left side)*. *Glycogenolysis* releases glucose units from glycogen. Debranching is first step in glycogenolysis *(right side)*.

Glucosyl residues are added to the glycogen primer by the formation of alpha-1,4-linkages. Glycogen grows by the sequential addition of glucosyl residues to form a straight chain. Eventually a branching enzyme known as amylo(1,4-1,6)transglucosylase will transfer a sequence of glucosyl units by breaking the alpha-1,4-linkage and re-attaching the sequence with an alpha-1,6-linkage. This branching enzyme moves a sequence of 6 or 7 glucosyl units from a chain that is at least 11 glucosyl units long and places the sequence at a branch point 4 units away from the nearest pre-existing branch. This branching, illustrated below leads to the most efficient storage of glucose.



As a result of the above reactions, glucose is stored as glycogen. The principle stores of glycogen occur in the liver and skeletal muscle.

GLYCOGENOLYSIS - THE DEGRADATION OF GLYCOGEN

Degradation of stored glycogen (glycogenolysis) occurs through the action of *glycogen phosphorylase*. The action of phosphorylase is to phosphorylytically remove single glucose residues from alpha-1,4-linkages within the glycogen molecules. The product of this reaction is glucose-1-phosphate. Glycogen phosphorylase removes one glucosyl residue at a time until it reaches 4 residues from a branch point. Steric hinderance prevents this enzyme from getting any closer to the branch point. Next, the enzyme *oligo-(1,4-1,4)-glucan transferase* picks up 3 of the 4 glucosyl residues and transfers these residues onto another chain making it long enough for glycogen phosphorylase to continue to remove glucosyl residues. Finally the action of *amylo-1,6-glucosidase* (also known as the debranching enzyme) catalyzes the hydrolysis of the one remaining glucosyl residue at the branch point forming glucose.

The next step involves the conversion of glucose-1-phosphate to glucose-6-phosphate by the enzyme *phosphoglucomutase,* the same enzyme that began this process. The glucose-6-phosphate may then continue through glycolysis (typical in skeletal muscle cells since there is no glucose-6-phosphatase available) or be converted to glucose (typical in the liver) to generate free glucose to maintain blood glucose levels.

HORMONAL REGULATION OF BLOOD GLUCOSE LEVELS

Reading - Kaplan page 587 beginning with the section entitled "Hormone Regulation of Glucose Metabolism" to page 589 ending where the section entitled "Glucose Metabolism in Diabetes Mellitus" begins.

As indicated in your reading, the concentration of glucose in the blood is normally maintained within a narrow range by hormones that modulate the movement of glucose into and out of the circulation. There are seven hormones that exert this effect.

1. **Insulin** - most of the assigned reading is related to insulin and this will be considered in more detail at a later time. At this time the main points to know about insulin is that it is produced by the beta cells of the Islets of Langerhans in the pancreas. Insulin promotes glycogenesis and lipogenesis. Therefore, insulin promotes the movement of glucose and lipids into cells for storage. The other six hormones that influence CHO metabolism tend to cause the opposite effects of insulin and are typically said to be antagonistic to insulin.
2. **Epinephrine** (also referred to by the older term adrenalin) - this is a catecholamine that is secreted by the adrenal medulla. Epinephrine stimulates glycogenolysis which results in an increased blood glucose level. Epinephrine stimulates glucagon secretion and inhibits insulin secretion. A tumor of the adrenal medulla is known as **pheochromocytoma**. This tumor will result in the increased production of epinephrine and norepinephrine which leads to increased blood glucose levels during this disease.
3. **Growth Hormone** - this is produced by the anterior pituitary gland (adenohyphophysis). Growth Hormone stimulates gluconeogenesis and lipolysis. Growth Hormone also antagonizes insulin-stimulated glucose uptake by cells.

4. **Cortisol** - this hormone is secreted by the adrenal cortex. Cortisol stimulates gluconeogenesis. Hyperactivity of the adrenal cortex is known as **Cushing's Syndrome** and results in the increased release of cortisol which leads to an increased blood glucose level. Dysfunction of the adrenal cortex is known as **Addison's Disease** and results in the decreased release of cortisol which leads to a decreased blood glucose level.
5. **Thyroxine** - thyroxine is produced by the thyroid gland. Thyroxine can stimulate glycogenolysis. In addition, thyroxine appears to increase the rate of gastric emptying and the intestinal absorption of glucose.
6. **Somatostatin** - this hormone is produced by the delta cells of the Islets of Langerhans in the pancreas. Somatostatin does not have a direct effect on CHO metabolism. Somatostatin is also called growth hormone inhibiting hormone and its inhibition of the release of growth hormone from the pituitary may indirectly lower blood glucose levels.
7. **Glucagon** - the effects of glucagon have already be considered during discussions of CHO metabolism. Glucagon is produced by the alpha cells of the Islets of Langerhans in the pancreas. Glucagon stimulates glycogenolysis and gluconeogenesis resulting in increased blood glucose levels. Glucagon secretion is primarily regulated by blood glucose levels.

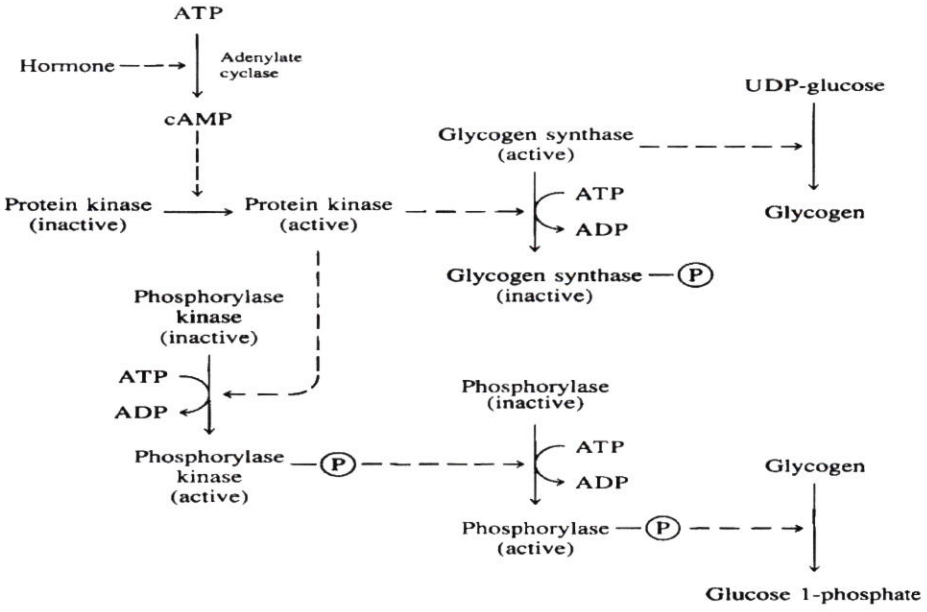
REGULATION OF GLYCOGEN METABOLISM

As seen with the regulation of glycolysis and gluconeogenesis, there must also be regulation of glycogen synthesis and glycogen degradation. Regulation of glycogen metabolism results from hormonal influences on the enzymes glycogen synthase and glycogen phosphorylase. When no hormonal stimulation is being received by the liver and/or skeletal muscle, glycogen synthesis occurs because glycogen synthase naturally exists in its active form and glycogen phosphorylase naturally exists in its inactive form. When there is a need for glucose to produce energy, the hormone glucagon will stimulate the liver to initiate glycogenolysis and the hormone epinephrine will stimulate the liver and skeletal muscle to initiate glycogenolysis. The effect of these hormones is to convert glycogen synthase to an inactive form (thus stopping glycogen synthesis) and to convert glycogen phosphorylase to an active form (thus initiating glycogenolysis).

For example, the following step-by-step process would occur when there is a need developed for glucose to be utilized for energy (refer to the diagram below).

1. The need for energy causes the release of glucagon and/or epinephrine.
2. These hormones will bind with their receptor on the surface of the liver and/or skeletal muscle cell.
3. This binding will activate a membrane-bound enzyme known as adenylate cyclase.
4. Adenylate cyclase will catalyze the conversion of ATP into cAMP (cyclic adenosine monophosphate).
5. cAMP will cause the activation of the enzyme protein kinase. (NOTE: protein kinase carries out two functions illustrated by dashed lines in the diagram below.)
6. The first action of protein kinase is to inactivate glycogen synthase. Protein kinase does this by catalyzing the phosphorylation of the active glycogen synthase forming inactive glycogen synthase. Thus, UDPG will not transfer glucosyl residues to glycogen.
7. The second action of protein kinase is to convert inactive phosphorylase kinase into active phosphorylase kinase by catalyzing the phosphorylation of this kinase.

8. The active form of phosphorylase kinase will catalyze the phosphorylation of inactive glycogen phosphorylase to active glycogen phosphorylase by catalyzing the phosphorylation of this enzyme. The active form of glycogen phosphorylase will then initiate glycogenolysis resulting in the formation of glucose-1-phosphate.
9. When the need for energy has been satisfied, there will be a decrease in hormonal stimulation. This will allow a phosphatase enzyme to hydrolyze the phosphorylated forms of glycogen phosphorylase, phosphorylase kinase, and glycogen synthase. Thus, glycogenesis will begin once again and glycogenolysis will be inhibited.



GLYCOGEN STORAGE DISEASES

Since glycogen molecules can become enormously large, an inability to degrade glycogen can cause cells to become pathologically engorged which can result in a functional loss of glycogen as a source of cell energy and as a blood glucose buffer. Although glycogen storage diseases are quite rare (for example, von Gierke's disease occurs at a rate of about 1 in 200,000 people), their effects can be quite dramatic.

Glycogen storage diseases result from genetic defects with one of the enzymes associated with glycogen metabolism. The table below summarizes the known glycogen storage diseases. Some characteristics of the more well known glycogen storage disease are described in the following paragraph.

Von Gierke's disease (Type I Glycogen Storage Disease) results from an absence of glucose-6-phosphatase. This results in hypoglycemia because glucose-6-phosphate cannot be hydrolyzed in the absence of this enzyme. As a result, glycogen accumulates in the liver which becomes massively enlarged. **Pompe's disease** (Type II Glycogen Storage Disease) results from an absence of oligo-(1,4-1,4)-glucan transferase. It is usually fatal by age two, as is **Andersen's disease** which is caused by a lack of amylo-(1,4-1,6)glucosyltransferase, the enzyme that adds branches to glycogen. The absence of skeletal muscle phosphorylase characterizes **McArdle's disease.** This defect is not life threatening, but persons suffering from this disease cannot perform strenuous exercise.

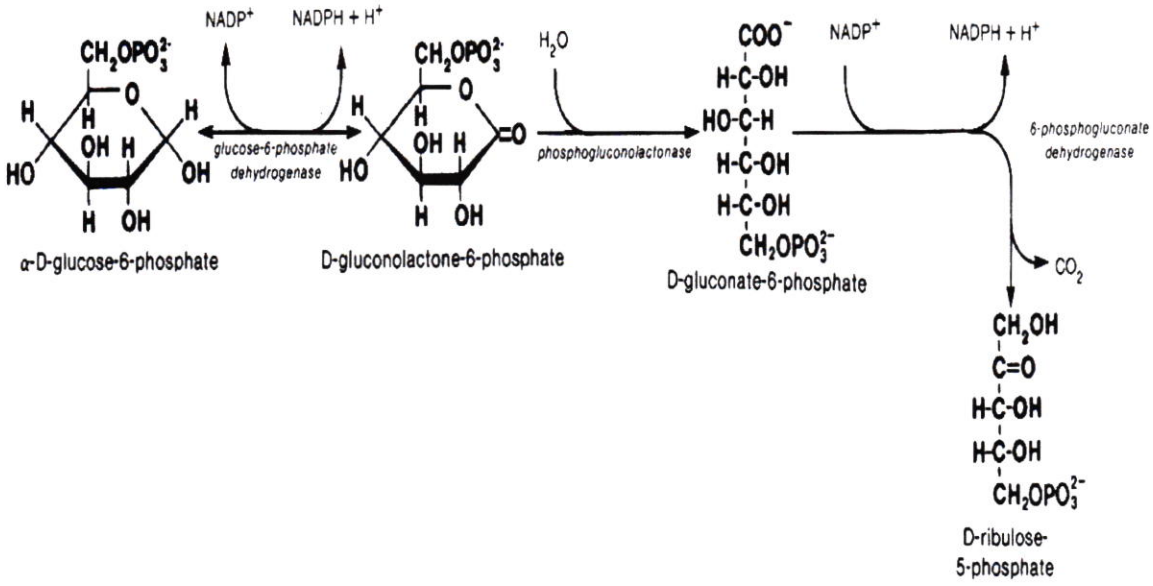
Glycogen Storage Diseases

Type	Defective Enzyme	Tissue Effected	Clinical Symptoms
I (Von Gierke's) (Illustrated in Fig 47-19, Page 928)	glucose-6-phosphatase	liver and kidney	Enlarged liver Failure to thrive Hypoglycemia
II (Pompe's)	oligo-(1,4-1,4)-glucan transferase	liver, heart, skeletal muscle	Cardiorespiratory failure Fatal by age 2
III (Cori's)	amylo-(1,6)-glucosidase	liver and skeletal muscle	Similar to Type I, but less severe
IV (Andersen's)	amylo-(1,4-1,6)-glucosyltransfersase	liver	Liver cirrhosis Fatal by age 2
V (McArdle's)	glycogen phosphorylase	skeletal muscle	Weak muscle
VI (Hers')	glycogen phosphorylase	liver	Similar to Type I, but less severe
VII	phosphofructokinase	skeletal muscle	Similar to Type V
VII	phosphorylase kinase	liver	Similar to Type I, but less severe

THE HEXOSE MONOPHOSPHATE SHUNT/PENTOSE PHOSPHATE PATHWAY

At the point of glucose-6-phosphate, metabolism may take another route. This route involves the Pentose Phosphate Pathway (PPP). The PPP is also known as the Hexose Monophosphate Pathway. The PPP has two primary goals.

1. Produce ribose-5-phosphate which serves as a precursor for nucleic acid and nucleotide synthesis.
2. Produce NADPH (reduced nicotinamide adenine dinucleotide phosphate), a phosphorylated form of NADH. This reducing power is used throughout the body which includes serving as a coenzyme for fatty acid and steriod biosynthesis.



Focusing on the production of NADPH, cells where biosynthesis of fatty acids and steroids are of importance, the PPP accounts for about 10 percent of the total glucose consumption. In other cells, such as skeletal muscle cells, this pathway is virtually non existent. NADPH is produced in two of the first three steps of the PPP as illustrated below.

Of particular interest is the production of NADPH in the erythrocyte. The predominate pathways of CHO metabolism in the erythrocyte are glycolysis, the 2,3-DPG shunt, and the PPP. Glycolysis provides ATP for erythrocyte function. The 2,3-DPG shunt is important in oxygen transport by hemoglobin. The PPP supplies the erythrocyte with NADPH to maintain glutathione in its reduced state.

Glutathione is a tripeptide composed of glutamate, cysteine and glycine. The reactivity of glutathione comes from the presence of the thiol (sulfhydryl) function making this compound an extremely important reductant. This reductant role of glutathione is extremely important in the highly oxidizing environment of the erythrocyte. The sulfhydryl group of glutathione can be used to reduce peroxides formed in the erythrocyte during oxygen transport. Peroxides (e.g. hydrogen peroxide - H_2O_2), if not removed, weakens the erythrocyte cell membrane and leads to hemolysis. Accumulation of peroxides will also lead to increased rates of oxidation of hemoglobin to methemoglobin. During the reduction of peroxides, glutathione becomes oxidized. The oxidized form of glutathione consists of two glutathione molecules joined at the sulfhydryl sulfur atoms by a disulfide bond. The reduced form of glutathione is abbreviated GSH and the oxidized form is abbreviated GSSG.

There is only a limited amount of glutathione in the erythrocyte. Thus, it is very important that oxidized glutathione be continually returned to its reduced state to offer optimal protection for the erythrocyte.

The reactions of glutathione are shown below. Reduced glutathione (GSH) utilizes the enzyme glutathione peroxidase to reduce peroxides to water. The result is the formation of oxidized glutathione (GSSG). GSSG is then reduced by the enzyme glutathione reductase. This enzyme requires NADPH as the coenzyme. It is this NADPH that is generated by the HMS. In fact, as much as 10% of the glucose consumption by the erythrocyte may be mediated by the HMS.

A deficiency of glucose-6-phosphate dehydrogenase in erythrocytes leads to acute hemolytic anemia following the intake of certain drugs. This drug induced anemia results from a genetic condition that effects about 11% of black Americans and more than 100 million people in central Africa. Glucose-6-phosphate dehydrogenase is essential for the proper function of erythrocytes because the NADPH produced by this enzyme is the reducing agent for glutathione reductase.

The gene for glucose-6-phosphate dehydrogenase is located on the X chromosome. If a male, who has only one X chromosome, inherits the mutation for glucose-6-phosphate dehydrogenase, he will be unable to synthesize normal enzyme. Inability to produce normal glucose-6-phosphate dehydrogenase is fatal. Females, who have two X chromosomes, may be either homozygotes, with two normal or two abnormal glucose-6-phosphate dehydrogenase genes, or heterozygotes, with one normal and one abnormal glucose-6-phosphate dehydrogenase gene. Heterozygotes have a partial deficiency of glucose-6-phosphate dehydrogenase.

The erythrocytes of heterozygotes for glucose-6-phosphate dehydrogenase deficiency are resistant to *Plasmodium falciparum*, a parasite that causes malaria. *Plasmodium falciparum* requires NADPH for growth. Erythrocytes of heterozygotes produce enough NADPH to support their own requirements but not enough to support optimum growth of the malarial parasite.

Certain antimalarial drugs, such as pamaquine and sulfur drugs and foods such as fava beans lower the concentration of NADPH in erythrocytes. Persons who produce normal amounts of glucose-6-phosphate dehydrogenase can maintain their erythrocyte NADPH concentrations at functional levels when treated with pamaquine, where those with decreased levels of G6P may develop an acute hemolytic anemia in response to the drug.

Summary - Regulation of Blood Glucose Levels

As we prepare to consider errors of CHO metabolism in the next Unit, a summary of the regulation of blood glucose levels is in order. This summary provides a means to expand on some of the concepts already presented.

If for no other reason, it is because of the demands of the brain for oxidizable glucose that the human body exquisitely regulates the level of glucose circulation in the blood.

Nearly all CHOs ingested in the diet are converted to glucose following transport to the liver. Catabolism of dietary or cellular proteins generates carbon atoms that can be utilized for glucose synthesis via gluconeogenesis. Additionally, other tissues besides the liver that incompletely oxidize glucose (predominately skeletal muscle and erythrocytes) provide lactate that can be converted to glucose via gluconeogenesis.

Maintenance of blood glucose homeostasis is of paramount importance to the survival of the human organism.

- The predominant tissue responding to signals that indicate reduced or elevated blood glucose levels is the liver. Indeed, one of the most important function of the liver is to produce glucose for the circulation.
- Both elevated and reduced levels of blood glucose trigger hormonal responses to initiate pathways designed to restore glucose homeostasis. **Low blood glucose triggers release of glucagon from pancreatic alpha cells. High blood glucose triggers release of insulin from pancreatic beta cells.**
- Additional signals, ACTH and growth hormone, released from the pituitary act to increase blood glucose by inhibiting uptake by extrahepatic tissues.
- Glucocorticoids also act to increase blood glucose by inhibiting glucose uptake.
- Cortisol, the major glucocorticoid released from the adrenal cortex, is secreted in response to the increase in circulating ACTH.
- The adrenal medullary hormone, epinephrine, stimulates production of glucose by activating glycogenolysis in response to stressful stimuli.
- Glucagon binding to its' receptors on the surface of liver cells triggers an increase in cAMP production leading to an increased rate of glycogenolysis by activating glycogen phosphorylase. This is the same response hepatocytes have to epinephrine release. The resultant increased levels of G-6-P in hepatocytes is hydrolyzed to free glucose, by glucose-6-phosphatase, which then diffuses to the blood. The glucose enters extrahepatic cells where it is re-phosphorylated by hexokinase. Since muscle and brain cells lack glucose-6-phosphatase, the glucose-6-phosphate product of hexokinase is retained and oxidized by the tissues.

- In opposition to the cellular responses to glucagon (and epinephrine on hepatocytes), **insulin stimulates extrahepatic uptake of glucose** from the blood and **inhibits glycogenolysis in extrahepatic cells** and **conversely stimulates glycogen synthesis**.
- As the glucose enters hepatocytes it binds to and inhibits glycogen phosphorylase activity. The binding of free glucose stimulates the de-phosphorylation of phosphorylase making it inactive.

Why is it that glucose that enters hepatocytes is not immediately phosphorylated and oxidized? Liver cells contain an isoform of hexokinase called glucokinase. Glucokinase has a much lower affinity for glucose than does hexokinase. Therefore, it is not fully active at the physiologic ranges of blood glucose. Additionally, glucokinase is not inhibited by its product, glucose-6-phosphatase, whereas, hexokinase is inhibited by G-6-P.

★ Hepatocytes, unlike most other cells, are freely permeable to glucose. Therefore, hepatocytes are essentially unaffected by the action of insulin.

★ When blood glucose levels are low, the liver does not compete with other tissues for glucose since the extrahepatic uptake of glucose is stimulated in response to insulin.

★ Conversely, when blood glucose levels are high, extrahepatic needs are satisfied and the liver takes up glucose for conversion into glycogen for future needs. Under conditions of high blood glucose, liver glucose levels will be high and the activity of glucokinase will be elevated. The glucose-6-phosphate produced by glucokinase is rapidly converted to glucose-1-phosphate by phosphoglucomutase, where it can be incorporated into glycogen.

KETOGENESIS

Ketogenesis is the production of ketones (also known by some as ketone bodies). **Ketogenesis begins at the point of acetyl-CoA**. Recall that acetyl-CoA is the point where CHO, protein and lipid metabolism all come together. Extrahepatic tissues almost exclusively utilize acetyl-CoA in the citric acid cycle. In the liver, acetyl-CoA may enter into other metabolic pathways as well as passing through the citric acid cycle. One of these other possible pathways in the liver is the conversion of acetyl-CoA into ketones.

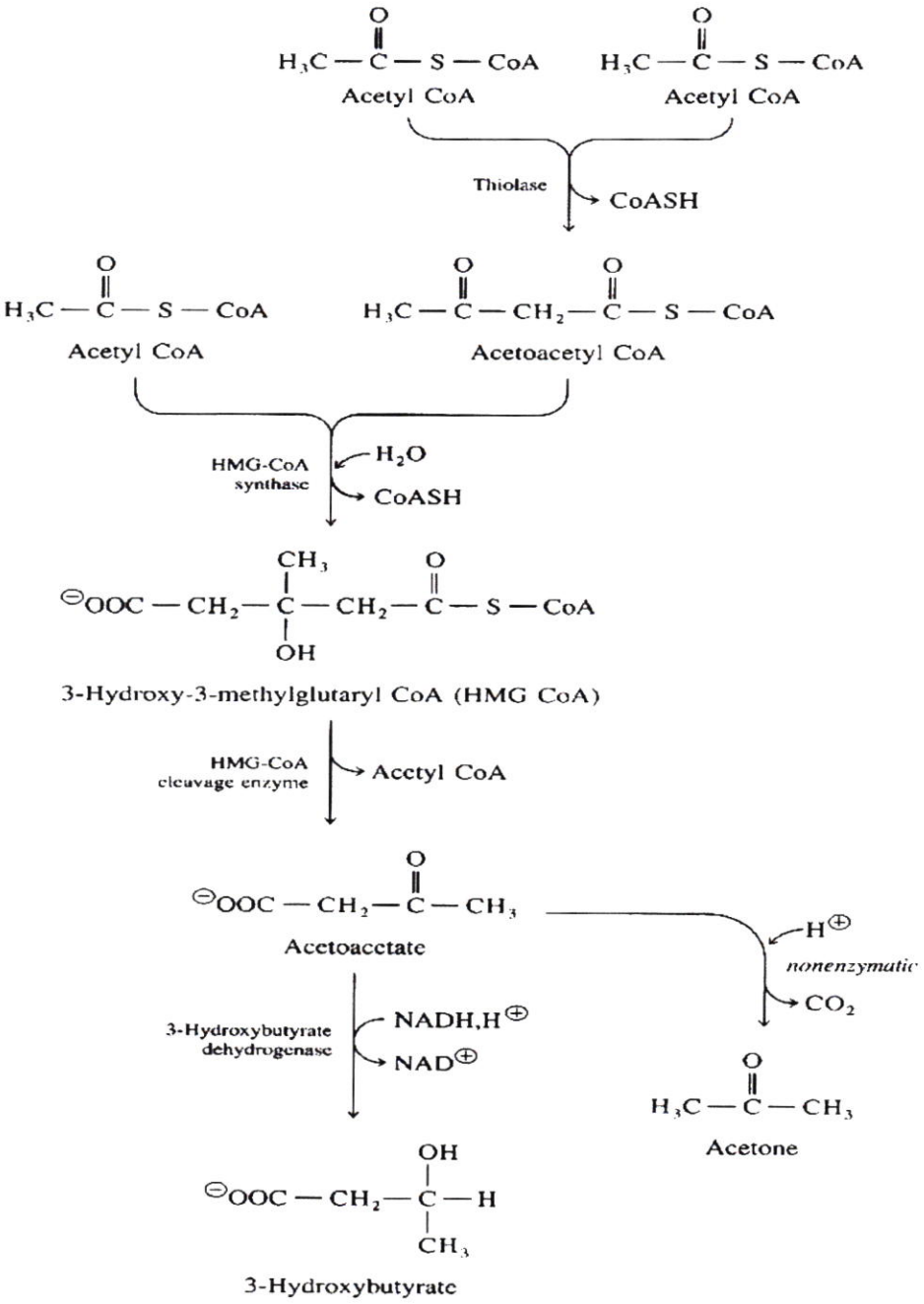
Significant ketone production occurs in situations such as starvation and diabetes mellitus. In these situations the following sequence of events occurs.

- This begins with a depletion of CHO inside the cell.
- This depletion results from no CHO being available in starvation and results from a lack of functional insulin in a case of diabetes. This insulin is necessary for glucose to enter many cells.
- Thus in diabetes mellitus we will find high blood glucose levels because the glucose cannot get into most cells for metabolism.
- As a result of the lack of available glucose to serve as an energy source, there will be a hormonal stimulus mobilizing fatty acids for metabolism (lipolysis).
- Fatty acids will be converted to acetyl-CoA. It would be anticipated that acetyl-CoA would then enter the citric acid cycle in order to produce needed energy. This will not happen however because there is an OAA deficiency preventing the citric acid cycle from turning.
- The OAA deficiency is a result of gluconeogenesis which is occurring at the same time as lipolysis.
- As a result acetyl-CoA accumulates.

- In the liver, the primary organ for ketogenesis, when acetyl-CoA levels begin to accumulate, the enzyme acetyl-CoA thiolase will condense two acetyl-CoA molecules and form acetoacetyl-CoA.
- Acetoacetyl-CoA will next combine with a third acetyl-CoA in the presence of the enzyme beta-hydroxy-beta-methylglutaryl-CoA synthase to form beta-hydroxy-beta-methylglutaryl-CoA (HMG-CoA).
- Some of the HMG-CoA leaves the mitochondria, where it is converted to mevalonate (the precursor for cholesterol synthesis) by HMG-CoA reductase.
- HMG-CoA in the mitochondria is converted to acetoacetate by the action of *HMG-CoA lyase*.
- **Acetoacetate is the first of three physiologically significant ketones**. Acetoacetate may be enzymatically converted to **beta-hydroxybutyrate (the second physiologically significant ketone)** through the action of beta-hydroxybutyrate dehydrogenase.
- Acetoacetate may also, as discussed below, leave the liver and enter the blood stream for distribution.
- When this acetoacetate comes in contact with oxygen under high tension (pressure) in the lungs, some may be spontaneously decarboxylated to form **acetone (the third physiologically significant ketone)**.

Of these two pathways, the conversion of acetoacetate to beta-hydroxybutyrate is by far the most prominent. It is important to note that the medical staff in the Emergency Department are trained to detect the presence of acetone on a person's breath. This may serve as a indicator of diabetic ketoacidosis, especially when the patient is in a comatose or semi-comatose state upon arrival.

The synthetic pathway described above is illustrated below



There is a purpose for ketogenesis. The production of ketones occurs at a relatively low rate during normal feeding and under conditions of normal physiological status. Normal physiological responses to CHO shortages cause the liver to increase the production of ketones from the acetyl-CoA generated from fatty acid oxidation. Once acetoacetate and beta-hydroxybutyrate are formed in the liver they are released into the blood stream for distribution.

Ketones are utilized by extrahepatic tissues through the conversion of beta-hydroxybutyrate to acetoacetate and of acetoacetate to acetoacetyl-CoA. The first step involves the reversal of the beta-hydroxybutyrate dehydrogenase reaction, and the second involves the action (shown below) of *acetoacetate:succinyl-CoA transferase*, also called *ketoacyl-CoA-transferase*.

Acetoacetate + Succinyl-CoA <------> Acetoacetyl-CoA + succinate

The latter enzyme is present in all tissues except the liver. Importantly, its absence allows the liver to produce ketones but not to utilize them. This ensures that extrahepatic tissues have access to ketone bodies as a fuel source during prolonged starvation.

The heart and skeletal muscles are the major tissues that use ketones for energy, thereby preserving the limited glucose for use by the brain. The brain also has the capability to acquire a limited capacity for oxidizing ketones after approximately three weeks of fasting as a protective measure.

Along with a beneficial effect of ketones goes a potential problem. When the production of ketones exceeds the capacity to use them, ketone levels build up in the blood stream. This is known as ketosis. Acetoacetate and beta-hydroxybutyrate are acidic. Thus, as the levels of these two ketones increase, there is the potential to develop acidosis. This problem will be seen as diabetic ketoacidosis in uncontrolled diabetes mellitus and is potentially life threatening.