MOLECULAR PRINCIPLES, COMPONENTS, TECHNOLOGY, AND CONCEPTS: METABOLISM

Contents

Metabolic Regulation

MS Patel, University at Buffalo, The State University of New York, Buffalo, NY, USA
RA Harris, Indiana University School of Medicine, Indianapolis, IN, USA

Glossary

Acetyl-CoA/CoA ratio An index of the acetylation potential of the cell. Set at different ratios in the cytosol and mitochondrial matrix space and varies with nutritional state. It is usually determined in the mitochondrial matrix space by the relative rates of fatty acid oxidation and citric acid cycle.

Allosteric control A control mechanism by which a small molecule affects one or more of the kinetic parameters of an enzyme by binding to a site on the protein other than the active site. Positive allosteric effectors increase enzyme activity. Negative allosteric effectors decrease enzyme activity.

Cellular compartments Spaces enclosed by biological membranes create cellular compartments. These include the cytosol which is enclosed by the plasma membrane and the mitochondrial matrix space which is enclosed by the mitochondrial inner membrane. These compartments serve as a way to separate metabolic pathways that involve common intermediates, for example, fatty acid oxidation in the mitochondrial matrix space and fatty acid synthesis in the cytosol.

Covalent modification A control mechanism by which covalent modification of the side chain of an amino acid residue of an enzyme affects the kinetic parameters of that enzyme. Depending upon the enzyme and the modification, the effect can have a positive or negative effect on enzyme activity. Common covalent modifications include phosphorylation, acetylation, acylation, succinylation, and methylation.

Energy charge An index of the energy status of cells. It is defined as ([ATP] + ½ [ADP])/ ([ATP] + [ADP] + [AMP]). Energy charge values range from 0 (all AMP) to 1.0 (all ATP). Cells maintain the energy charge within the narrow range of 0.8 to 0.95 by modifying rates of ATP producing and ATP utilizing reactions.

Isozymes or isoenzymes Enzymes that are different gene products but catalyze the same reaction, for example, HK 1, 2, 3, and 4 all catalyze the reaction of glucose + ATP yields G6P + ADP. Isozymes invariably differ with respect to their kinetic characteristics (K_m, V_{max}) and regulatory mechanisms (sensitivity to allosteric effectors, whether subject to covalent modification, whether regulated by gene transcription).

Malate/aspartate shuttle Cyclic pathway that transports reducing equivalents in the form of NADH from the cytosol into the mitochondrial matrix space (mitosol). The sum reaction for the cycle is NADHcytosol + NAD^+mitosol yields NAD^+cytosol + NADHmitosol.

NAD^+/NADH ratio An index of the redox state of the cell. Set at different ratios in the cytosol and mitochondrial matrix space and varies with the nutritional state. It is usually determined in the mitochondrial matrix space by the relative rates of fatty acid oxidation and the electron transport chain.

Oxidative phosphorylation The process by which the energy requiring reaction of ATP synthesis from ADP and Pi is coupled to the energy producing pathway of electron transport.

Phosphorylation potential An index of the energy status of cells. It is calculated from the equation [ATP]/[ADP][Pi] which derives from the free energy change for ATP hydrolysis. Accurate determination requires estimation of the free concentrations of the nucleotides and inorganic phosphate. Cells maintain very high phosphorylation potentials to drive high energy requiring anabolic processes.

Tricarboxylic acid (TCA) cycle, Krebs cycle, citric acid cycle Cycle that oxidizes acetyl-CoA to CO_2 with the production of NADH and FADH2 which in turn are oxidized by the electron transport chain with the production of ATP by oxidative phosphorylation. It is named after Hans Krebs who discovered the citric acid cycle.

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Introduction

Metabolic regulation is a term used to describe the process by which metabolic pathways (both the anabolic/biosynthetic and catabolic/degradative pathways) are regulated in mammals. Living organisms need to generate energy continuously to maintain cellular processes and functions. The ability to oxidize available substrates (termed as fuels) to maintain energy needs (energy homeostasis) is central to survival of an organism. In mammals a near constant level of blood glucose (glucose homeostasis) is maintained to supply this fuel for energy production by the brain and other tissues. Hence, the maintenance of energy homeostasis and glucose homeostasis is critical for the function and survival of mammals during the fed as well as the fasting states (due to intermittent consumption of dietary fuels). Insulin and glucagon produce opposite effects upon metabolic processes. Caloric homeostasis in the fed and the fasted states depends upon a continuous monitoring and adjustment of the blood concentrations of insulin and glucagon. A change in the flux in a metabolic pathway is achieved by modulating the activity of one or more key enzymes (regulatory enzymes) which are subject to a variety of mechanisms to control their activities. Thus, by modulating the flux through various metabolic pathways to meet the metabolic needs of different organs, the body is able to maintain both its energy homeostasis and glucose homeostasis by utilizing the available fuels either from the dietary sources or from the internal tissue deposits.

The purpose of this article is to provide a summary of well-established principles of metabolic regulation in cells and tissues of animals. Rather than an exhaustive description of what is known about the regulation of a particular enzyme or a metabolic pathway, we discuss metabolic regulation in the context of physiological conditions that will be of interest to most readers. In our experience as investigators in this field as well as teachers of medical biochemistry, we have found that the remarkable ways in which the liver is able to change from an organ that synthesizes fuels for storage in the fed state to an organ that provides fuels for the rest of the body is a good way to gain an appreciation of the different mechanisms by which metabolic pathways are regulated (Figure 1).

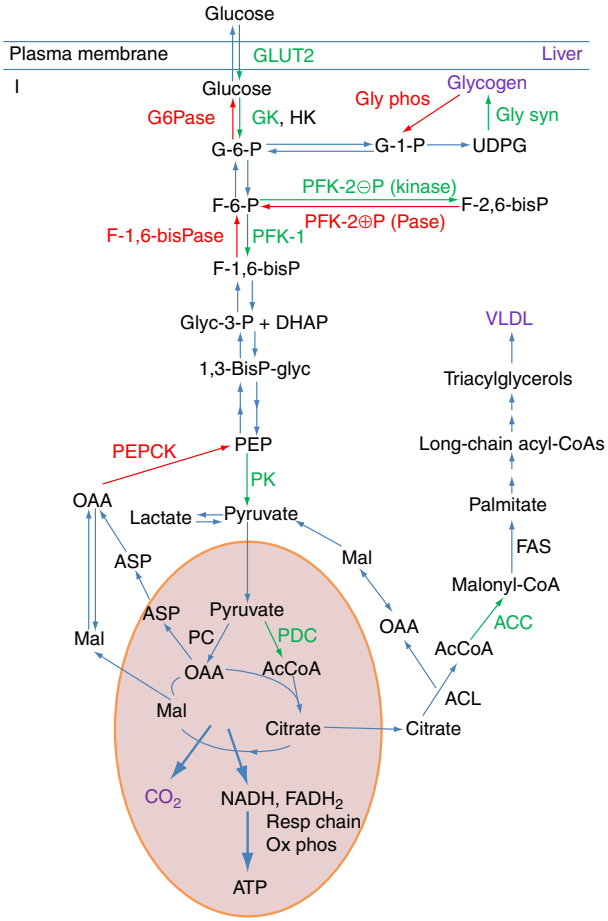

Figure 1 The pathways involved in glucose metabolism in the liver during the fed and fasted states. The major pathways depicted here are: glycolysis, glycogen synthesis and degradation, the tricarboxylic acid cycle, lipogenesis, and gluconeogenesis. These pathways are abbreviated to highlight the key regulatory enzymes. The key enzymes and the transporter in the biosynthetic pathways in the liver during the fed state are shown in green color and the key enzymes in the degradative pathways operating in the fasted state are in red color. Enzyme abbreviation used are: HK, hexokinase; GK, glucokinase; GLUT2, glucose transporter 2; Gly syn, glycogen synthase; Gly phos, glycogen phosphorylase; PFK-1, phosphofructokinase-1; PFK-2, phosphofructokinase-2; F-1,6-bisPase, fructose-1,6-bisphosphatase; PK, pyruvate kinase; PDC, pyruvate dehydrogenase complex; PC, pyruvate carboxylase; ACL, ATP-citrate lyase; ACC, acetyl-CoA carboxylase; FAS, fatty acid synthase. Abbreviations used for intermediates are commonly used in the metabolic pathways.

Mechanisms for Regulation of Key Enzymes

Before we discuss the alterations in the pathways involved in glucose metabolism in the liver during the fed and fasted states, the mechanisms involved in controlling the rates of key regulatory enzymes need to be discussed. Although a change in the overall flux in a given pathway determines the contribution of that pathway in the metabolism in tissues, the control of this change in the flux is achieved by regulating key enzymes in the given pathway (usually more than one). This control of key enzymes can be achieved by five different mechanisms.

Substrates (Fuel) Availability

In the fed state, markedly increased availability of the circulating fuels (glucose, amino acids, and triacylglycerols) along with the increased levels of circulating insulin set the stage for anabolic metabolism in all tissues. In the fasted state, a change in the levels of circulating hormones favoring the action of glucagon signals for mobilization (as glucose, free fatty acid, glycerol, lactate, pyruvate, and amino acids) of the stored fuels in the forms of glycogen in the liver, triacylglycerols (TAGs) in adipose tissues and proteins in skeletal muscle. Several enzymes and transporters have high Kms for their substrates and hence they operate mainly when the concentration of the required substrates is increased (e.g., hepatic glucokinase, an isoenzyme of hexokinase, when the portal level of glucose is markedly increased during absorption after a mixed meal).

Allosteric Modulation

A change in enzyme activity by an allosteric mechanism brought about by a conformational change in the enzyme by binding of a small molecule in the site other than the active site. This type of enzyme is generally a dimer or higher order of organization. The change in activity (activation or inhibition) occurs as rapidly as the level of an allosteric compound changes in the cell and hence is referred to as short-term control.

Covalent Modification (or Posttranslational Modification)

A covalent modification refers to attachment/removal of a group (such as phosphoryl, acetyl, etc.) to/from a specific amino acid residue on an enzyme/protein. This change results in either activation or inactivation of enzyme activity. A change in enzyme activity by a covalent modification (e.g., phosphorylation of a serine residue on an enzyme by a posttranslation modification) is rapid (short-term control) with often all-or-none activity of a modified enzyme molecule. This is probably the most effective way to regulate enzyme activity.

Interconversion of a key regulatory enzyme between its two forms (active and inactive) by covalent modification imparting different kinetic and allosteric properties often provides not only a unique control for its regulation but also by its interactions with a group of key enzymes involved in the biosynthetic and degradative pathways involved in a given metabolic process (e.g., protein–protein interactions among phosphorylase kinase, glycogen phosphorylase, and glycogen synthase in glycogen metabolism).

Inhibitory Protein (Regulatory Protein) Interaction

Binding of an inhibitory protein (aka regulatory protein) to an enzyme interferes with enzyme action. For example, binding of glucokinase inhibitory protein to glucokinase inhibits its activity. Similarly, binding of a specific inhibitory protein (aka regulatory protein) to protein kinase A (catalytic protein) inactivates protein kinase A. When cAMP binds to the inhibitory protein, it dissociates from protein kinase A making the latter an active enzyme.

Transcriptional and Degradational Control

A change (either increase or decrease) in the transcriptional rate of a gene by its transcriptional induction or repression eventually results in an alteration in the level of enzyme content (actual number of enzyme molecules) per cell. This is a relatively slow response requiring hours if not days for a significant response (and hence referred to as a long-term control). This control mechanism modulates the metabolic capacities of tissues over a longer time period in days and weeks under a given dietary condition. With some enzymes, levels are also regulated by degradation of the steady state amount of the protein.

Metabolism in the Fed State

An average American diet for adults provides approximately 2500 kcal day^{-1} as percentage of calories from three major components: 40–50% from carbohydrates, 35–40% from fats, and 12–15% from proteins. This level of calories per day is commonly ingested in the form of three meals supplemented with a couple of small snacks. Hence each meal (initiating the fed state) provides a larger excess of calories than what can be utilized immediately for the cellular needs. The fed state refers to the time period following the consumption of a meal in which the glucose, amino acids, and fatty acids derived from the carbohydrate, protein, and fat in the diet are being absorbed from the intestinal tract and distributed in the body by the blood. The excess of the consumed calorie as fuels need to be converted into storage forms such as glycogen in liver (Figure 1) and skeletal muscle and TAGs in adipose tissues. Absorptive state initiated after ingestion of a mixed meal causes transient increases in plasma glucose, amino acids, and TAGs (as chylomicrons and very low density lipoproteins called VLDL). Increased levels of glucose and amino acids in the plasma stimulate insulin secretion and diminish glucagon secretion by the endocrine pancreas, resulting in an increased molar ratio of insulin:glucagon in the plasma in the absorptive state. This change in the hormonal levels sets the direction of anabolic metabolism in all tissues and lasts for about 2–4 h depending upon the size and composition of a meal.

Action of Insulin

The binding of insulin to its receptor (a protein tyrosine kinase) on the plasma membrane results in the activation of a series of protein kinases that increase the enzymatic capacity for glucose uptake, glycogen synthesis, and fat synthesis (Figure 2). Glucose uptake in skeletal muscle and adipose tissue is increased by stimulating the translocation of glucose transporter 4 (GLUT4) loaded intracellular vesicles to the plasma membrane (Figure 2). The synthesis of glycogen is increased in liver and skeletal muscle by activation of glycogen-bound phosphatases (such as protein phosphatase-1, PP-1) that activate glycogen synthase by dephosphorylation (Figure 2). The synthesis of fat is increased in liver and adipose tissue by promoting gene transcription and subsequent synthesis of lipogenic enzymes that include acetyl-CoA carboxylase (ACC) (Figure 1), fatty acid synthase (FAS), malic enzyme (ME), and fatty acid desaturases (Figure 1). Furthermore, there is increased VLDL synthesis and secretion by the liver (Figure 1), and increased synthesis of TAGs in adipose tissues as well as deposition of TAG derived from chylomicrons and VLDL in adipose tissue to store excess calories derived from dietary carbohydrates and fats. Also, protein synthesis is enhanced in all tissues especially in the skeletal muscle. The major tissues of interest in this metabolic interplay are liver, skeletal muscle, adipose tissues, and the brain. To illustrate the major regulatory mechanisms, we will discuss the metabolism of glucose by the liver during the fed state.

Glucose Metabolism in the Liver (in the Fed State)

Glucose transport and Its phosphorylation

It is no wonder that the liver is considered a processing plant for handling of excess fuels for storage in the fed state and

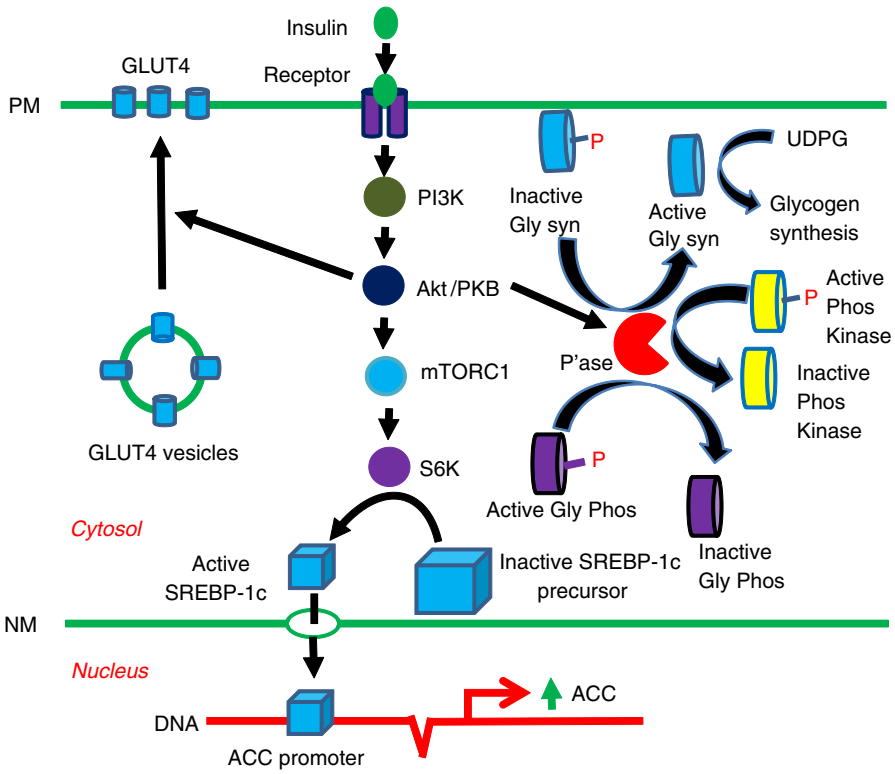

Figure 2 Insulin promotes glucose uptake, glycogen synthesis, and fatty acid synthesis in tissues. The insulin signaling pathway involves activation of PI3K (phosphoinositide 3-kinase), Akt which is also known as PKB (protein kinase B), mTORC1 (mammalian target of rapamycin complex 1), and S6K (S6 ribosomal protein kinase). Akt/PKB promotes activation of glycogen-associated protein phosphatases (probably both PP1 and PP2A) which activate Gly syn (glycogen synthase) and inactivate Phos kinase (phosphorylase kinase) and Gly Phos (glycogen phosphorylase) by dephosphorylation in the liver and skeletal muscle. Akt/PKB also promotes the translocation of vesicles with membrane-bound GLUT4 (glucose transporter 4) from cytosol to the plasma membrane in skeletal muscle and adipose tissues where they fuse and thereby increase the capacity for glucose uptake from the blood. By a mechanism that is a subject of current research, S6K activates proteolytic processing of SREBP-1c (sterol response element binding protein 1c) to produce an active SREBP-1c peptide fragment that binds to the promoter for ACC (acetyl-CoA carboxylase) and promotes ACC transcription. Increased ACC expression along with increased expression of several other lipogenic enzymes increases the rate of fatty acid synthesis in the liver and adipose tissue.

manufacturing/supplying the fuels to the rest of the body during the fasting state. Liver possesses two unique glucose sensors, namely glucose transporter 2 (GLUT2) and glucokinase (both with high Km for glucose) (Agius, 2008). Hence the transport of glucose in hepatocytes is increased via GLUT2 and its phosphorylation to glucose-6-phosphate is enhanced by glucokinase (in addition to hexokinase with a low Km, <0.1 mM, for glucose present in hepatocytes). Hence glucokinase activity increases rapidly as glucose concentration in the liver rises (via blood supply by the portal vein) during the absorptive state. Since glucokinase is not inhibited by its reaction product (in contrast to hexokinase), it allows rapid phosphorylation of glucose to glucose-6-phosphate for its metabolism via different pathways. Interestingly, activity of glucokinase is inhibited by its interaction with glucokinase regulatory protein in the presence of fructose-6-phosphate (which is in equilibrium with glucose-6-phosphate by the action of phosphoglucose isomerase) and this inhibition is overcome by fructose-1-phosphate formed from fructose metabolism (from dietary source as sucrose) in the liver (not shown in figure).

Regulation of glycogen synthesis

Glucose-6-phosphate is readily utilized for the synthesis and storage of glycogen and its metabolism is enhanced to pyruvate via the glycolytic pathway due to the action of several regulatory enzymes under the control of insulin-mediated actions. Acetyl-CoA generated from pyruvate via the action of the pyruvate dehydrogenase complex (PDC) is largely diverted for the synthesis of long-chain fatty acids and cholesterol for the formation of VLDL, and is also oxidized via the tricarboxylic acid (TCA) cycle to generate ATP to support the biosynthetic processes, as indicated. These biosynthetic pathways are enhanced by activation of several key regulatory enzymes by their dephosphorylation (covalent modification) (**Figure 2**) as well as the changes in the levels of allosteric modulators (**Table 1**). Dephosphorylation of these regulatory enzymes is achieved by the activation of phosphoprotein phosphatase-1 (PP-1) mediated by the action of insulin (**Figure 2**) (Roach *et al.*, 2012).

Glycogen synthase is the regulatory enzyme in the pathway of glycogen synthesis and is subject to regulation by three mechanisms listed above. The phosphorylated form of glycogen synthase (also referred to as 'D' form for Dependent form;

Table 1 Regulation of several key enzymes in glucose metabolism by their phosphorylation status and allosteric modifiers in the liver

Enzyme	*Compounds (+ activator/– inhibitor)*	*Active enzyme (phosphorylation status)*
Glycogen synthase	+ Glucose-6-P + Glucose – ATP, – ADP, – Pi	Non-phospho form
Phosphorylase kinase		Phospho form
Glycogen phosphorylase	– Glucose – ATP – Glucose-6-P	Phospho form
Phosphofructokinase-1	+ Fructose-2,6-bisP + AMP – ATP, – Citrate	
Phosphofructokinase-2 (kinase)		Non-phospho form
Phosphofructokinase-2 (phosphatase)		Phospho form
Pyruvate kinase	+ Fructose-1,6-bisP	Non-phospho form
Pyruvate dehydrogenase complex		Non-phospho form
Pyruvate dehydrogenase kinases	+ Acetyl-CoA + NADH – Pyruvate, – CoA – ADP, – NAD^+	
Acetyl-CoA carboxylase	+ Citrate – Acyl-CoA	Non-phospho form
Pyruvate carboxylase	+ Acetyl-CoA	
Fructose-1,6-bisphosphatase	– Fructose-2,6-bisP – AMP	

'inactive' form or glycogen synthase 'b') is strongly inhibited by physiological concentrations of ATP, ADP, and Pi, but this inhibition can be overcome by glucose-6-phosphate. A non-phosphorylated 'I' form (Independent form or 'active form' or glycogen synthase 'a' form) does not require glucose-6-phosphate for its activity. PP-1, activated by the action of an insulin-dependent protein kinase, dephosphorylates the phosphorylated form of glycogen synthase ('D' form; inactive form) into an 'active' form ('I' form) (**Figure 2**), resulting in an increased flux through this enzyme. Binding of glucose (above 7 mM) to glycogen phosphorylase 'a' promotes its conversion from 'a' form to inactive 'b' form, resulting in its inactivation (and hence inhibiting glycogen degradation). Dephosphorylation of phosphorylase kinase and glycogen phosphorylase (**Figure 2**) results in the conversion of their 'active' forms into 'inactive' forms. Inactivation of glycogen phosphorylase results in release of PP-1 which then dephosphorylates phosphorylated glycogen synthase 'D' (inactive form) to its non-phosphorylated 'I' form (active form) stimulating glycogen synthesis (**Figure 2**).

PP-1 is active when it is associated with glycogen through its glycogen-binding G protein. G protein is subject to phosphorylation at two distinct sites: (1) phosphorylation of site 1 by an insulin-stimulated protein kinase activates PP-1 and (2) phosphorylation of site 2 (and also of site 1) by cAMP-dependent protein kinase A (PKA) results in its dissociation from glycogen-associated enzyme complex. In the cytosol, PP-1 inhibitor binds to PP-1, rendering it inactive. Interestingly, PP-1 inhibitor is also subject to phosphorylation (activation) by PKA and dephosphorylation (inactivation) by PP-1 (Roach *et al.*, 2012).

Furthermore, for a long-term regulation of glycogen synthase an intracellular signaling pathway mediated by insulin enhances transcription of the glycogen synthase gene, resulting in increased synthesis of this enzyme protein.

Regulation of the glycolytic pathway

The liver has a limited capacity to store glucose as glycogen (80–100 g) and hence the excess glucose-6-phosphate is processed via the glycolytic pathway and the hexose-monophosphate (pentose) pathway to generate eventually acetyl-CoA and NADPH to support lipid biosynthesis in the liver. The glycolytic pathway has two regulatory enzymes, namely phosphofructosekinase-1 (PFK-1) and pyruvate kinase (**Figure 1**). PFK-1 (the first committed enzyme in the glycolytic pathway) is subjected to allosteric regulation by a number of molecules (namely fructose-2,6-bisphosphate, AMP, ATP, citrate, etc.). Among these allosteric modulators of PFK-1, fructose-2,6-bisphosphate, a product of phosphofructokinase-2 (PFK-2) derived from fructose-6-phosphate, is the most potent activator of PFK-1 (**Figure 3**) (Kurland and Pilkis, 1995). Interestingly, PFK-2 (which is a bifunctional enzyme with alternate functional kinase or phosphatase activity) is regulated by its covalent modification. Dephosphorylated PFK-2 protein possesses kinase activity, resulting in increased synthesis of fructose-2,6-bisphosphate which then acts as an activator of PFK-1 (**Figure 3**). In contrast, PFK-2 in its phosphorylated form expresses phosphatase activity and causes a breakdown of fructose-2,6-bisphosphate to fructose-6-phosphate (hence lowering the intracellular concentration of fructose-2,6-bisphosphate) (**Figure 3**). Hepatic PFK-1 is also subject to long-term regulation at the transcriptional level. Activity of

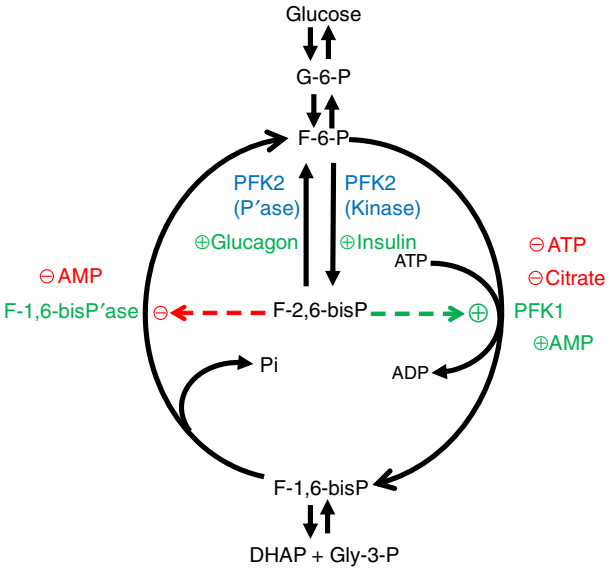

Figure 3 Regulation of glycolysis and gluconeogenesis at the level of PFK1 and F-1,6-bisP'ase. PFK1 uses ATP to convert F-6-P to F-1,6-bisP in the glycolytic pathway. F-1,6-bisP'ase catalyzes the hydrolysis of F-1,6-bisP to F-6-P in the gluconeogenic pathway. Flux in the direction of glycolysis is greatly increased by allosteric activation of PFK1 by F-2,6-bisP coupled with allosteric inhibition of F-1,6-bisP'ase by this same compound. PFK2 is a bi-functional enzyme that functions as a kinase for the conversion of F-6-P to F-2,6-bisP in its dephosphorylated state and as a phosphatase for the conversion of F-2,6-bisP back to F-6-P in its phosphorylated state. By signaling the dephosphorylation and therefore the activation of the kinase moiety of PFK2, insulin increases the level of F-2,6-bisP which stimulates glycolysis and inhibits gluconeogenesis. By signaling the phosphorylation (through PKA) and therefore the activation of the phosphatase moiety of PFK2, glucagon decreases the level of F-2,6-bisP which inhibits glycolysis and stimulates gluconeogenesis. AMP acts as an allosteric effector in manner similar to F-2,6-bisP, that is, it activates PFK1 and inhibits F-1,6-P'ase and ATP opposes stimulatory effect of AMP on PFK1. On the other hand, citrate allosterically increases the effectiveness of ATP as an allosteric inhibitor of PFK1.

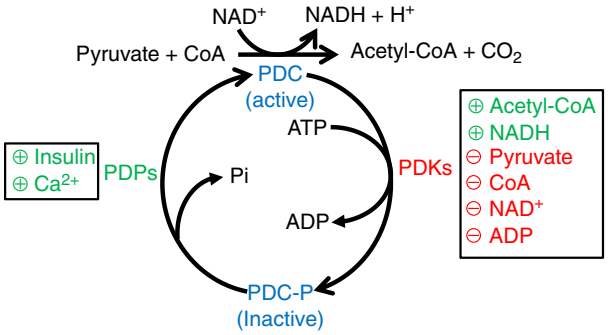

Figure 4 Regulation of the pyruvate dehydrogenase complex. PDC, a multi-enzyme complex found in the mitochondrial matrix space, is responsible for the oxidation of pyruvate to acetyl-CoA and CO_2 with concurrent reduction of NAD^+ to NADH. Subject to regulation by covalent modification, PDC is inactive in phosphorylated state catalyzed by PDKs (pyruvate dehydrogenase kinases) and active in the dephosphorylated state catalyzed by PDPs (pyruvate dehydrogenase phosphatases). Positive allosteric effectors of PDKs are given in green and negative allosteric effectors in red. One of the PDPs is allosterically activated by Ca^{2+}. Although the mechanism remains to be defined, insulin stimulates PDC activity by promoting PDP activity. PDKs and PDPs are integral proteins of the PDC complex.

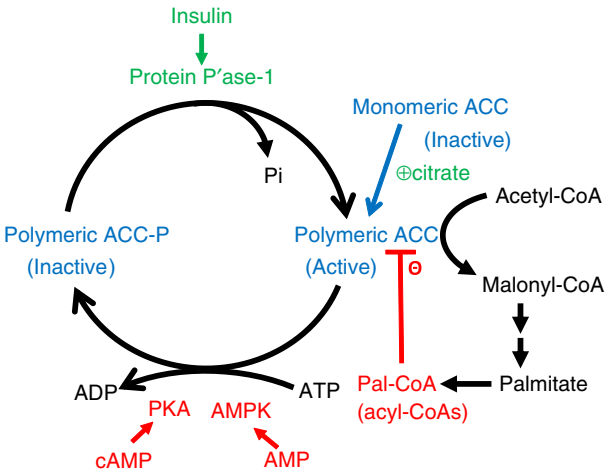

Figure 5 Regulation of fatty acid synthesis at the level of acetyl-CoA carboxylase. ACC is responsible for the conversion of acetyl-CoA to malonyl-CoA for fatty acid synthesis. It exists in the liver cytosol as inactive monomers and large active polymers that are subject to inactivation by phosphorylation by PKA and AMP kinase (AMPK). By increasing cAMP, glucagon promotes inactivation of ACC and therefore inhibition of fatty acid synthesis. Likewise, an energy shortage that causes a fall in ATP and consequently an increase in AMP will also inactivate ACC and inhibit fatty acid synthesis. By signaling the activation of protein phosphatase-1 (PP-1), insulin promotes dephosphorylation and therefore activation of ACC and stimulates fatty acid synthesis. Allosteric inhibition of ACC by long-chain acyl-CoA esters is a good example of regulation of a pathway (lipogenesis) by feedback inhibition.

pyruvate kinase is subject to regulation by all three mechanisms. Fructose-1,6-bisphosphate is an allosteric activator of (a feed-forward stimulation in the pathway) of liver pyruvate kinase (Table 1). This enzyme is also subject to a phosphorylation/dephosphorylation mechanism. Dephosphorylation of pyruvate kinase by PP-1 results in the conversion to its active form. Insulin-mediated transcription of the pyruvate kinase gene also plays a role in maintaining a steady state level of this enzyme in the liver. Collectively, the increased activities of three enzymes, namely glucokinase, PFK-1 and pyruvate kinase contribute to an increased flux in the glycolytic pathway (glycolysis) in the liver during the fed state.

Regulation of the pyruvate dehydrogenase complex

Pyruvate generated from glucose via the glycolytic pathway is transported by a carrier into the mitochondria where it is metabolized to acetyl-CoA and oxaloacetate by the PDC and pyruvate carboxylase, respectively. Dephosphorylation of phospho-PDC by its specific pyruvate dehydrogenase phosphatases results in its activation resulting in increased formation of acetyl-CoA for the formation of citrate (Figure 4) (Harris *et al.*, 2002; Patel and Korotchkina, 2003). Acetyl-CoA

also allosterically activates pyruvate carboxylase to increase oxaloacetate synthesis for citrate formation. A small portion of citrate is metabolized to CO_2 in the TCA cycle to form NADH and FADH2 which are further processed via the respiratory chain and the oxidative phosphorylation pathway to generate ATP. The terms, the energy charge and phosphorylation potential, are generally used to calculate energy status of cells.

Regulation of fatty acid synthesis

The bulk of citrate is transported out to the cytosol to regenerate acetyl-CoA by the action of ATP-citrate lyase (dephosphorylated 'active' form). Acetyl-CoA in the cytosol is utilized for the synthesis of long-chain fatty acids (palmitate) (**Figure 1**) and cholesterol. ACC is the first committed step in the pathway of fatty acid synthesis, and is also subject to regulation by the three mechanisms (**Figures 2** and **5**). ACC in its monomeric form is inactive. Citrate enhances the formation of a polymeric, active form (a chain-like conformation) (**Figure 5**). Dephosphorylation of a polymeric form is the most active form and its dephosphorylation is carried out by PP-1 (**Figure 5**). For its long-term regulation, its gene transcription is up-regulated by the action of insulin-mediated signaling pathway (**Figure 2**). Activation of ACC increases the formation of malonyl-CoA which is further utilized by fatty acid synthase for the biosynthesis of long-chain fatty acids, palmitate (**Figure 1**). For the reductive synthesis of fatty acids, NADPH is also required. This requirement is met by the action of three enzymes, namely glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, and NADP-malate dehydrogenase (aka malic enzyme) in the cytosol. The protein levels of these enzymes are regulated transcriptionally by the action of insulin (Owen *et al.*, 2012; Zoncu *et al.*, 2011). Increased levels of malonyl-CoA also act as an allosteric inhibitor of carnitine acyl-CoA transferase-I (McGarry, 1998) so that newly synthesized fatty acids (after conversion into their CoA derivatives) are not transported into mitochondria for oxidation but rather utilized for the synthesis of TAGs and incorporated into VLDL for transport. The separation of the pathway for fatty acid synthesis in the cytosol from the pathway of fatty acid oxidation in the mitochondria is a good example of cellular compartmentation for achieving metabolic regulation. The cytosolic pool of acetyl-CoA is also used for the synthesis of cholesterol in the liver. The key regulatory enzyme in this pathway is 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase which is subject to regulation by sterol-dependent regulation of its gene expression, sterol-accelerated enzyme degradation, covalent modification via a phosphorylation/dephosphorylation mechanism, and insulin-dependent up-regulation of its gene transcription. Hence, HMG-CoA reductase activity is increased in the fed state by its dephosphorylation and hence enhancing cholesterol synthesis in the liver. In the fed state, the newly synthesized TAGs and cholesterol are used for the synthesis of VLDL and transported into the blood. Liver also processes chylomicron remnants and reutilizes their TAGs for the formation of VLDL.

Protein synthesis

In the fed state, amino acids derived from dietary proteins are preferentially utilized for protein synthesis in the liver. Excess amino acids are catabolized and their nitrogen is directed for urea synthesis. Acetyl-CoA derived from the degradation of carbon-skeletons of amino acids can be utilized for ATP production and/or lipid synthesis in the fed state. This, however, may represent a minor contribution to the total hepatic lipid synthesis.

Metabolism in the Fasted (Starvation) State

Fasted (starvation) state can arbitrarily be subdivided in three stages: (1) early fasted state, (2) intermediate fasted (starvation) state, and (3) prolonged fasted (starvation) state. Early fasted state is initiated after the post-absorptive period and continues for about 24–48 h after the last meal. There are metabolic changes which take place in tissues to maintain energy homeostasis and glucose homeostasis during this period (Cahill, 2006). Towards the end of the post-absorptive state as plasma glucose gradually returns to its basal level ($\sim$5 mM) so is the level of plasma insulin. During the early fasting state as the plasma levels of glucose and insulin continue to decrease, the plasma level of glucagon rises resulting in a reduction in the molar ratio of circulating insulin: glucagon ratio which favors the action of glucagon at the cellular level.

Action of Glucagon

Glucagon via interaction with its receptors (a G-protein coupled receptor, GPCR) increases synthesis of cAMP by adenylate cyclase, resulting in increased activity of cAMP-dependent protein kinase (PKA) (**Figure 6**). Increased PKA activity causes phosphorylation of the key regulatory enzymes in the metabolic pathways in the tissues (Berglund *et al.*, 2009; Harris and Crabb, 2010). This action increases the liver's capacity for glucose production by glycogenolysis and gluconeogenesis (**Figures 1** and **6**). The overall effect of this covalent modification is glycogenolysis since phosphorylation activates the key enzymes localized in the catabolic pathways (e.g., phosphorylase kinase and glycogen phosphorylase 'a') in the glycogen degradation pathway in the liver (**Figure 6**), and hormone-sensitive lipase in the TAG degradation pathway in adipose tissues. Glucagon increases glucose synthesis by promoting gene transcription and subsequent synthesis of gluconeogenic enzymes that include phosphoenolpyruvate carboxykinase (PEPCK) (**Figure 6**), F-1,6-bisphosphatase (F-1,6-bisPase), and glucose-6-phosphatase (G-6-Pase) (**Figure 1**).

Additionally, phosphorylation of the key enzymes (e.g., glycogen synthase, pyruvate kinase, ACC, etc.) in the biosynthetic pathways (**Figure 6**) results in inactivation of these enzymes to avoid futile cycling. The carbon-fluxes via the biosynthetic pathways (such as glycogen synthesis, fatty acid biosynthesis, and cholesterol synthesis) are markedly inhibited due to phosphorylation and hence inactivation of the key enzymes in these pathways (**Figure 1**). Protein synthesis is also markedly down regulated by reduction in insulin-mediated actions. Hence, this change in the plasma hormonal levels sets the direction from anabolic (biosynthetic mode) to catabolic state (degradative mode) in all tissues.

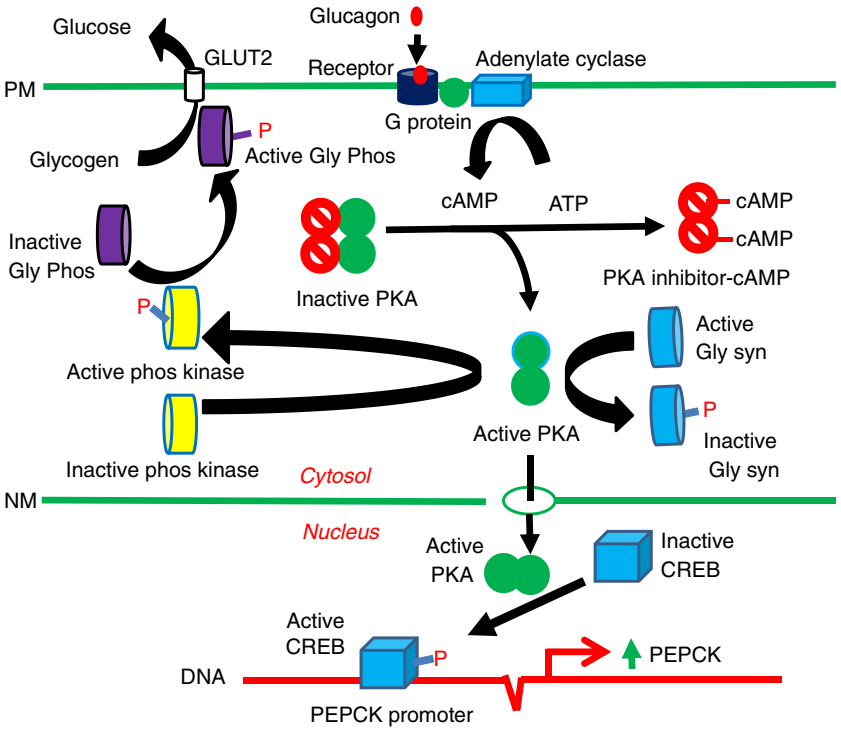

Figure 6 Glucagon promotes glucose production by glycogenolysis and gluconeogenesis. The glucagon signaling pathway involves activation of adenylate cyclase to produce cAMP which activates PKA (protein kinase A) by releasing an inhibitory protein. Active PKA promotes glycogen degradation by inactivating Gly syn (glycogen synthase) and activating Phos kinase (phosphosphorylase kinase) by phosphorylation. Active phosphorylase kinase activates Gly Phos (glycogen phosphorylase) by phosphorylation. Active PKA moves into the nucleus where it activates CREB (cAMP-responsive element-binding protein) by phosphorylation. Active CREB binds to the promoter of the PEPCK gene to promote transcription of the gluconeogenic enzyme PEPCK. Increased expression of PEPCK along with increased expression of other gluconeogenic enzymes increases the rate of gluconeogenesis.

Metabolism during Prolonged Starvation

After this period (~48 h) fasting extends into an intermediate starvation state lasting for about 2 weeks with major metabolic shifts in tissue fuel choices (Cahill, 2006). During this state the levels of plasma ketone bodies gradually rise to very high levels (in the range of 5 mM), resulting in increased transport and oxidation by the brain (due to high Kms for ketone bodies by the monocarboxylic acid transporter). This shift allows the brain to meet its energy needs (to maintain energy homeostasis) but reducing its total dependency on glucose oxidation. This reduction in glucose demand by the brain coincides with reduction in the rate of hepatic gluconeogenesis from its precursors such as amino acids, glycerol, and lactate and pyruvate. Reduction in hepatic gluconeogenesis reflects in reduction in proteolysis in the skeletal muscle, a sparing effect on protein degradation. Selective fuel utilization in the brain oxidative metabolism from primarily glucose-dependent to a state of mixed fuel dependency (glucose plus ketone bodies) allows humans to survive for a much longer period of total starvation with a limited level of available protein mass (to support gluconeogenesis) and TAG mass in adipose tissues (to support energy homeostasis). During the prolonged starvation state (beyond about 2 weeks of starvation), this adaptive metabolic profile of the brain continues to operate with ketone bodies serving as major fuel (approximately two-third of the energy needs) and glucose oxidation providing the rest. If starvation continues in this period, a continued demand on body fat and protein reserves cannot be met, leading to a catastrophic condition as death if feeding is not gradually initiated.

The normal American meal-eating pattern results in a re-occurring cycle of absorptive, post-absorptive and early fasted states. In a modern life-style with easily available foods, fasting beyond 24–48 h can only occur by predetermined reasons such as medical, religious, and occasionally political causes. Hence, in this section we will limit discussion on liver metabolism to the early fasting (starvation) state during the first 24–48 h.

Glucose Metabolism in the Liver (During Fasting)

Regulation of glycogen degradation

During the early fasted state the rates of glucose utilization by most tissues decrease (except the brain and tissues with anaerobic metabolism), and the rate of glucose release by the liver increases steadily due to breakdown of stored glycogen. The liver is the sole source of glucose provider for the rest of the body. The contribution to glucose release from hepatic glycogen gradually decreases after about 12 h of fasting and it is compensated by a gradual increase in the rate of hepatic gluconeogenesis during this period. After about 24 h of

fasting, liver glycogen is nearly depleted and the rate of glucose output by the liver is primarily supported by hepatic gluconeogenesis to maintain whole body glucose homeostasis. The rate of hepatic glycogenolysis is increased by phosphorylation (and hence activation) of its two regulatory enzymes, namely phosphorylase kinase and glycogen phosphorylase 'a' (**Figure 6**) (Roach *et al.*, 2012). These two enzymes are covalently modified by PKA which phosphorylates (and hence activates) phosphorylase kinase 'b' form to its 'a' form. Phosphorylase kinase 'a' then phosphorylates glycogen phosphorylase converting its 'b' (inactive) form into 'a' (active) form (**Figure 6**). This two-step activation cascade enhances rapid release of glucose from stored glycogen. Activities of these enzymes are also influenced by small molecules such as glucose, glucose-6-phosphate, and other compounds (allosteric effectors) (**Table 1**). Glucose-1-phosphate generated by this degradative pathway is first converted to glucose-6-phosphate and then to free glucose by glucose-6-phosphatase for its release from the liver.

Regulation of gluconeogenesis

Sources of gluconeogenic precursors are derived from different metabolic pathways: (1) lactate, pyruvate, and alanine are largely derived from inhibition of glucose-derived pyruvate oxidation by the PDC due to the metabolites (NADH and acetyl-CoA) generated from β-oxidation of fatty acids in the skeletal muscle, (2) glycerol generated from TAG breakdown by adipose tissues, and (3) release of gluconeogenic amino acids from proteolysis in the skeletal muscle. The carbon-skeletons of these amino acids are utilized for glucose synthesis and their amino-nitrogen is used for the synthesis of urea for nitrogen disposal. During the early state of fasting (about 24 h), all three sources contribute to glucose synthesis and its release by the liver.

Increases in hepatic cAMP levels also increase transcription of the PEPCK gene (**Figure 6**) (Yang *et al.*, 2009; Lin and Accili, 2011), resulting in a rapid accumulation of its enzyme protein within a few hours. Increased PEPCK activity together with increased availability of gluconeogenic precursors (such as lactate, pyruvate, and amino acid carbon-skeletons) results in an increased carbon-flux through this initial reaction in the gluconeogenic pathway (mitochondrial oxaloacetate to malate or aspartate and their transport to the cytosol via the malate/aspartate shuttles) for conversion to oxaloacetate and then to phosphoenolpyruvate (**Figure 1**). Phosphorylation and hence inactivation of hepatic pyruvate kinase in the cytosol and the PDC by its specific pyruvate dehydrogenase kinases (PDKs) in the mitochondria (**Figure 4**) direct the carbon-flux in the gluconeogenic pathway by inhibiting the former enzyme and minimizing the oxidation of the 3-carbon compound, pyruvate, by the latter enzyme complex (Harris *et al.*, 2002; Patel and Korotchkina, 2003). Additionally, phosphorylation of PFK-2 by PKA converts this bifunctional enzyme from its kinase activity to its phosphatase activity (**Figure 3**). PFK-2 (phosphatase) activity dephosphorylates fructose-2,6-phosphate to fructose-6-phosphate and hence lowers its intracellular concentrations and reduces its inhibition on fructose-1,6-phosphatase, a gluconeogenic enzyme, converting fructose-1,6-phosphate to fructose-6-phosphate. Glycerol derived from lipolysis in adipose tissues is phosphorylated to glycerol-3-phosphate and then converted to dihydroxyacetone phosphate, a glycolytic intermediate. The final step in the gluconeogenic pathway is glucose-6-phosphatase which converts glucose-6-phosphate to glucose for its release. A long-term regulation of this enzyme is exerted at its transcriptional level.

Fatty acid oxidation and ketogenesis

This early fasting state is also characterized by increased mobilization of non-esterified fatty acids from TAGs breakdown (lipolysis) in adipose tissues. This results in increased levels of plasma non-esterified fatty acids, transported on serum albumin, which contribute to the energy demands (energy homeostasis) of liver, skeletal muscles, kidneys, heart, and other tissues by increased oxidation of fatty acids. This process also suppresses glucose oxidation and hence contributes to the maintenance of glucose homeostasis (Randle, 1998). Fatty acid oxidation via the β-oxidation pathway generates acetyl-CoA and reduced nucleotides (NADH and FADH2), resulting in altered intramitochondrial acetyl-CoA/CoA ratio and also NAD^+/NADH ratio, supporting gluconeogenesis and ketogenesis. Re-oxidation of these nucleotides via the respiratory chain and the oxidative phosphorylation pathway generates ATP to support gluconeogenesis. Acetyl-CoA acts as an allosteric activator of pyruvate carboxylase, an anaplerotic enzyme, converting pyruvate to oxaloacetate which is then converted to phosphoenolpyruvate by PEPCK. Both Acetyl-CoA and NADH act as activators of PDK (**Table 1**) (Harris *et al.*, 2002; Patel and Korotchkina, 2003) and cause phosphorylation/inactivation of PDC to conserve pyruvate for gluconeogenesis. During this period the liver also initiates the synthesis of ketone bodies (ketogenesis) from acetyl-CoA generated from fatty acid oxidation via the β-oxidation pathway. Ketone bodies are released by the liver, and are readily oxidized by several tissues (such as skeletal muscle, kidneys, heart, etc.) but not by the brain (due to its transporters with high Kms for ketone bodies). The plasma levels of ketone bodies are maintained at a relatively low level (about 1–2 mM) during this early fasting state.

Dedication

This article is dedicated to Dr. Richard W. Hanson of Case Western Reserve University School of Medicine for his devotion and enormous contributions in the area of regulation of gluconeogenesis, for passionate teaching of metabolism, and for mentoring the next generation of investigators in the area of metabolic regulation. One of us (MSP) deeply acknowledges Dr. Hanson's generous mentorship, collegiality, and friendship over a period of 45 years.

See also: Molecular Principles, Components, Technology, and Concepts: Basic Principles: Biocatalysis. Molecular Principles, Components, Technology, and Concepts: Carbohydrates: Glycogen and Starch. Molecular Principles, Components, Technology, and Concepts: Metabolism: A Structure Perspective on Organelle Bioenergetics

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