Chapter 9
Regulation and Integration of Metabolism During Hypoglycemia

In humans, blood glucose is the main reporter of fed and fasting states. Its concentration directly regulates the secretion of hormones, including glucagon and insulin by the pancreas and glucocorticoids by the adrenal cortex. These hormones, in a coordinated action, regulate the energy metabolism in different organs, allowing the blood concentration of glucose to be maintained within a narrow range by a precise system balancing glucose production by the liver and its utilization by peripheral tissues.

The basis for this major role of glucose in controlling human metabolism may be discussed in the context of human evolution. About 4 million years ago, carbohydrates were important components of the diet of primates and prehuman ancestors, which possibly favored brain and reproductive tissues to develop a specific requirement for glucose as their primary fuel. The subsequent periods of human evolution were dominated by severe Ice Ages that selected the hominids who developed hunting and fishing abilities and consumed high-protein and low-carbohydrate diets. This led to metabolic adaptations to protect the brain and embryonic tissues from the low-glucose availability, resulting in the increased efficiency of hepatic glucose production and the decrease in peripheral glucose utilization. Additionally, the alternate periods of food scarcity and abundance selected metabolic mechanisms to increase deposition of energy reserves during periods of plenty for subsequent use when food was not available, favoring lipid accumulation and the development of the adipose tissue. However, the advent of agriculture after the last Ice Age greatly modified the quantity of carbohydrates consumed by humans, and the Industrial Revolution in the nineteenth century dramatically changed the quality of the carbohydrate ingested (see Box 9.1). These events together would result in an increase of postprandial glycemia and insulinemia, probably contributing to the predisposition of the modern diseases known as metabolic syndrome (see Chap. 11).

Nowadays, in Western cultures, humans have three main meals per day, and the amount of carbohydrates in a regular meal is about 50–60 %. After the digestion and absorption of the carbohydrates, glucose reaches the bloodstream and glycemia rapidly rises from the basal value of 4 mM to around 10 mM (see Fig. 8.2). In Chap. 8,

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we discussed the biochemical mechanisms that explain why plasma glucose concentration sharply falls down between the first and the second hour after a meal.

In this chapter, we will turn our attention to the subsequent hours, which are characterized by a slow decrease in glycemia. We will focus on the metabolic pathways and the mechanisms involved in the maintenance of blood glucose concentration when carbohydrates are not ingested, which include the periods in between meals as well as during low-carbohydrate diets or prolonged starvation. Additionally, the metabolic interrelationships that take place in the different organs and the hormonal regulation in this situation will also be discussed.

Box 9.1: The Evolution of Human Diet

Paleolithic and current Western diets show profound differences in composition, especially regarding carbohydrate content and quality (see figure). Several studies suggest that two major dietary shifts occurred during human evolution: (a) in the Neolithic (~10,000 years ago), when agriculture emerged, leading to an increase in the consumption of domesticated cereals and the adoption of carbohydrate-rich diet, and (b) after the Industrial Revolution (~1850), with the introduction of industrially processed flour and sugar. This led to the hypothesis, proposed in a classical article published by Eaton and Konner in 1985, that predisposition of the modern diseases known as metabolic syndrome, resulted from an evolutionary discordance between the adaptations established in the Paleolithic era (2.6 million to 12,000 years ago) and the way of life in the industrialized world. Based on this, the authors proposed the "Paleolithic diet" as a reference for human nutrition. However, this view is being questioned nowadays, especially because (a) it implies that human genetic background has not changed since Paleolithic, although humans continued evolving in the Neolithic period, with genetic changes directly related to diet variations, such as on the genes that code for amylase (enzyme that degrades starch) production, and (b) it does not take into account the non-genomic form of inheritance, such as the epigenetic regulation of gene expression, which alters fitness in short-term environmental shifts, such as during in utero development.

The dietary impacts during human evolution can be exemplified with one interesting study that analyzed the genetic diversity of oral microbiota of calcified dental plaque obtained from prehistoric European human skeletons, including the remains of the last hunter-gatherers in Poland and the earliest farming culture in Europe (the Linear Pottery Culture, LBK), as well as late Neolithic (Bell-Beaker culture), early and later Bronze Age, and medieval rural and urban populations (see in the figure an example of phylum frequencies obtained comparing a specific genome region). This study revealed that oral microbiota became markedly less diverse in the modern times, with the dominance of the potentially cariogenic bacteria, like *S. mutans* (see figure).

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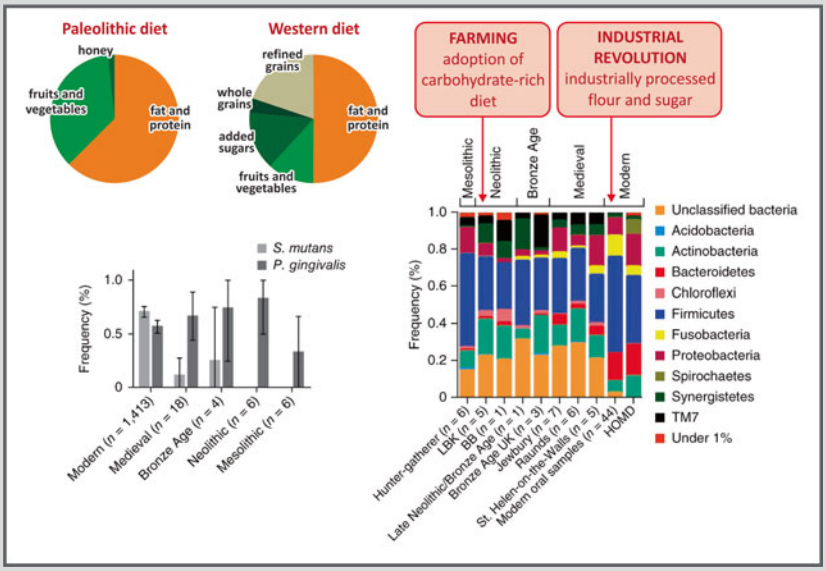
Box 9.1 (continued)



Carbohydrate consumption by man's ancestors corresponds to about 35 % of the total energy intake, which comes mainly from fruits and vegetables, while 50 % daily energy in a Western diet is obtained from carbohydrate, of which about 15 % comes from sugar added to food during processing or consumption, and about 20 % comes from refined cereals (Based on data from Eaton SB. Proc. Nutr. Soc. 65:1–6, 2006). The changes in carbohydrate content and quality in human diet impact in the diversity of oral microbiota (Reproduced by permission from Macmillan Publishers Ltd: Adler et al., Nat. Genet. 45:450–456, 2013)

The maintenance of glycemia may be explained by two distinct phenomena: the decrease in glucose utilization by different tissues and the increase in its production and release into the bloodstream by the liver and kidney (Fig. 9.1). When plasma glucose concentration is above the basal levels, insulin secretion is stimulated and the action of this hormone allows for rapid glucose utilization by all the tissues (see Chap. 8). However, as glycemia decreases, glucagon secretion will predominate, leading to a complete change in metabolism, which is characterized by a decrease in glucose utilization, especially by the muscle and adipose tissues, and a continuous release of glucose in the bloodstream by the liver and, in smaller quantities, by the kidney cortex, as we will discuss in the next sections.

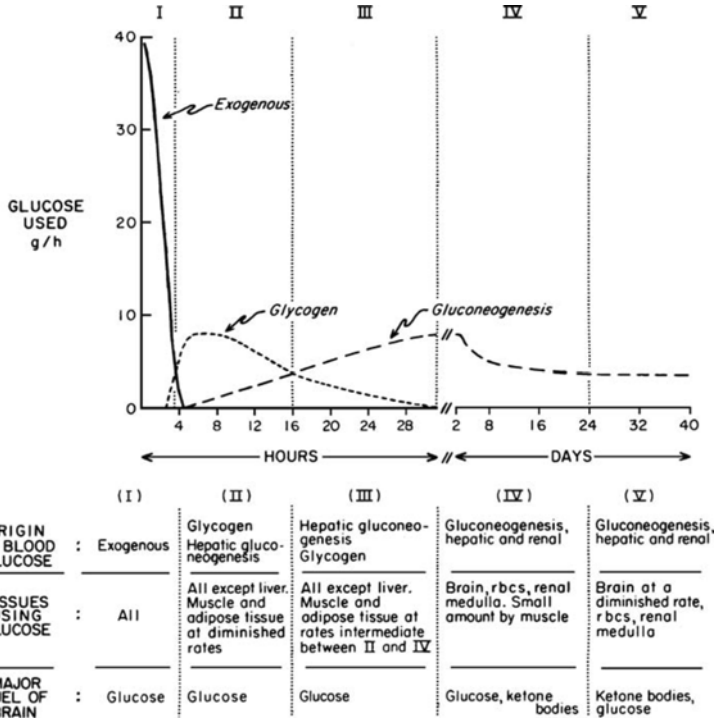


Fig. 9.1 Classical figure by Dr. George Cahill in which he describes five metabolic stages between the postabsorptive state and the near-steady state of prolonged starvation. The figure was constructed based on studies performed by Dr. Cahill and his group with patients submitted to therapeutic starvation in the 1960s (see Box 9.2). *rbcs* red blood cells (Reproduced with permission from Cahill. Ann. Rev. Nutr. 26:1–22, 2006)

9.1 Overview of Metabolism During Fasting: Exemplifying with Studies on Therapeutic Starvation

To introduce the issue of glycemia control, we will take advantage of some studies carried out between the 1950s and 1960s, when therapeutic starvation was used as a strategy to treat obesity (Box 9.2). It is important to note that this extreme situation was chosen as an example for clarity, but the adaptations and the metabolic pathways that will be discussed along this chapter also occur in more common situations, such as low-carbohydrate diets, overnight fasting, or even during the periods in between meals.

Box 9.2: Therapeutic Starvation

The studies used here to exemplify the metabolic adaptations to hypoglycemia have been performed by the group of Dr. George F. Cahill (1927–2012) during the 1960s. As pointed out by Richard W. Hanson in a retrospective article on Dr. Cahill's contributions to the understanding of human metabolism, the fact that he was not a biochemist but a physician–scientist made his approach to research integrative and not reductionist in nature. This integrative view allows him to make a crucial discovery regarding brain metabolism during fasting that explained how humans can survive for more than 60 days without food: ketone bodies, molecules derived from fatty acid metabolism, supply most of the energy requirements of the brain during fasting (see details in Sect. 9.3.4). This finding "resulted in a total reappraisal of the hierarchy of fuels used by different tissues of humans," as stated by Dr. Oliver E. Owen, who worked with Dr. Cahill on these classical experiments. In the 1950s and 1960s, therapeutic starvation of obese subjects was in vogue. The studies described here were performed in the Peter Bent Brigham Hospital, which has a National Institutes of Health-supported clinical research center where the patients were housed and continuously observed during experimental protocols. The patients spent 5–6 weeks fasting with total withdrawal of calories, when the daily intake consisted of one multivitamin capsule, water, and salt replacement. Dr. Owen tells in one of his articles that when someone asked him why he chose a 6-week period for starvation, he answered citing St. Matthew 4:2: "Jesus fasted forty days and forty nights and afterward he hungered." During treatment, the patients volunteered for blood and urine collections for measurements of the plasma concentrations of different metabolites. Furthermore, some of them underwent catheterization to determine the consumption or the production of metabolites by different organs by measuring the arterial-venous differences of these substances. Some of the data originated from these studies will be used in this chapter to discuss several aspects of human adaptation to fasting.

The variation of blood concentration of different metabolites during fasting will be used as the starting point for our discussion on the metabolic adaptations to hypoglycemia (Fig. 9.2).

As seen in Fig. 9.2, the concentration of fatty acids in the blood, after a slight increase, remains constant during all the period of fasting due to the equilibrium between its use as energy source by many tissues and its mobilization from triacylglycerol stored in the adipose tissue. Indeed, the content of triacylglycerol stored in the human body may provide energy for approximately 2 months of fasting (Table 9.1).

As ketone bodies are the main product of fatty acid oxidation in the liver (see Sect. 7.4.6), it is expected that the concentration of these metabolites increases

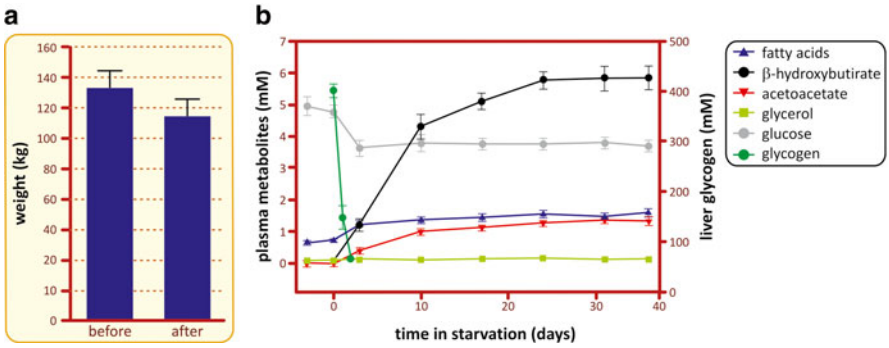


Fig. 9.2 (**a**) Average weight of a group of 11 obese patients before and after they were subjected to the treatment at the Clinical Center of the Peter Bent Brigham Hospital. (**b**) Concentrations of different metabolites in the plasma and glycogen in the liver during fasting (Based on data from Owen et al. J. Clin. Invest. 48, 574–583, 1969)

Table 9.1 Human nutrient stores (typical of a 70 kg man)

Molecule	Weight (g)	Energetic value (kcal)	Period as single energy source (days)
Triglyceride (adipose tissue)	9,000–15,000	~108,000	60
Glycogen (liver)	90	360	0.2
Glycogen (muscle)	250	1000	0.55
Glucose (blood and body fluids)	20	80	0.044
Proteins (mainly muscle)[a]	8000	32,000	17.8

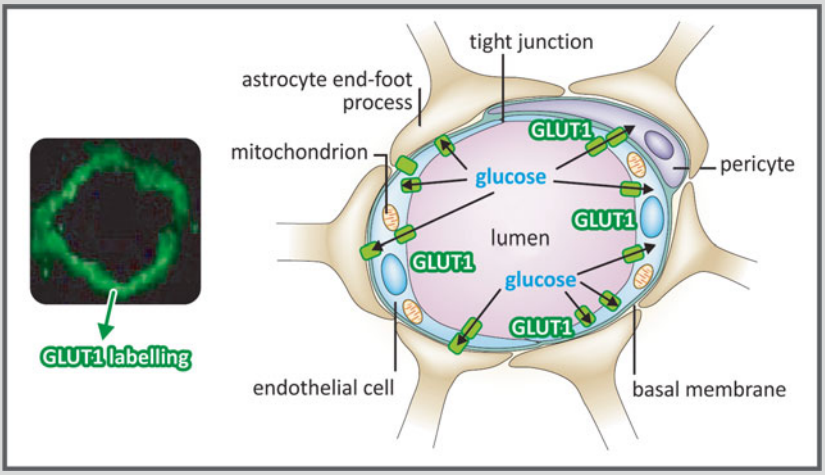
[a]It should be stressed that most of the muscular proteins are not readily available for mobilization

along fasting, as observed in Fig. 9.2. It is possible to note two different phases in the profile of the blood concentration of ketone bodies. In the first 10 days of fasting, there is a constant increase in their concentration, which then gradually reaches a plateau after 25 days. This second phase may be explained by the increase in ketone bodies' consumption and/or excretion by the organism. In fact, brain adaptation to the use of ketone bodies as its main source of energy contributes to their removal from the bloodstream as well as decreasing whole body glucose requirements (for more details, see Fig. 9.16).

A remarkable observation regarding the profile of plasma metabolites during fasting is that glucose concentration, after a small decrease in the first 3 days, is maintained constant during all the period of starvation. From this observation, immediate questions arise: Why is it necessary to maintain glycemia? Why not use only fatty acids as the energy source during fasting since they are, quantitatively, the major fuel reserve in the body (see Table 9.1)? The answer lies in the fact that some cells depend on glucose as their exclusive or preferential source of energy. This is the case of cells that lack mitochondria, as erythrocytes and the cells from crystalline lens, which depend on the anaerobic metabolism (glucose fermentation, see Sect. 6.1) to survive. It is also the case of the cells from the nervous system and

the embryonic tissues, which are isolated from the systemic circulation by blood barriers that do not allow the uptake of fatty acids since these molecules are bound to albumin, their major form of transport in the bloodstream (Box 9.3). Therefore, it is essential that glucose is constantly produced and released in the bloodstream during the periods in which it is not ingested. The metabolic pathways involved in the maintenance of glycemia will be discussed in the next sections.

Box 9.3: Glucose Transport Through the Blood–Brain Barrier (BBB)
BBB is a highly selective permeability barrier that restricts the passage of most substances from the circulating blood to the central nervous system (CNS) fluids (see also Sect. 3.3.4.1). It is formed by three cellular components (see figure): (a) the brain capillary endothelial cells; (b) the astrocytic end feet, which cover the vessel wall maintaining the endothelial barrier; and (c) the pericytes. The brain capillary endothelial cells are connected by tight junctions that make the paracellular transport of substances through BBB negligible under physiological conditions. Additionally, these cells express a number of drug efflux transporters, such as the glycoprotein P (Pgp) and several members of the multidrug resistance (MDR) protein family, which prevent the entry or remove drugs and other substances from the CNS. Glucose transport through BBB is mediated by GLUT1 (see Sect. 8.1). It is important to remind that GLUTs are facilitated-diffusion transporters, which means that glucose cannot be transported against a gradient from bloodstream to CNS. Additionally, GLUT1 K_M for glucose transport is about 1–2 mM. Thus, the human brain cannot be supplied with glucose when its blood concentration is low (normoglycemia is about 5 mM), so that the only strategy to protect brain cells from starvation is to prevent hypoglycemia.



Micrograph of a brain microvessel section showing GLUT1 labeled with a green fluorescent probe (*left*) and the schematic representation of the BBB with its three cellular components (*right*), highlighting the glucose transport through GLUT1 (represented as *green rectangles*). (Reproduced by permission from Macmillan Publishers Ltd: Löscher & Potschka. Nature Rev Neurosci 6:591–602, 2005)

9.2 Glycogen Degradation in the Liver

The crucial role of liver metabolism in producing glucose to maintain glycemia started to be elucidated in the middle of the nineteenth century, with the pioneering studies developed by Claude Bernard (Box 9.4).

In 1853, Claude Bernard showed that the liver was able to release glucose in the bloodstream even when carbohydrate was not present in the diet. This finding completely changed the current idea about animal nutrition. It contradicted the accepted concept that animals always decompose complex substances obtained from food and for the first time suggested that organism functions would be maintained by a metabolic interplay among different tissues. Some years later, Claude Bernard isolated from the liver a substance that he named "la matière glycogène" (the substance that generates glucose), the glycogen.

At that time, it was not yet clear that glucose was released from the liver by a combination of two processes: the degradation of glycogen and the synthesis of glucose from noncarbohydrate precursors. Glycogen may be seen as a transient storage of the ingested sugar that is mobilized when necessary, while different non-glycidic

Box 9.4: Claude Bernard: The Founder of Experimental Medicine
Maybe the most important accomplishment of Claude Bernard was to introduce the scientific methodology into medicine and physiology, establishing the basic rules of experimentation in the life sciences. This can be noticed in one of this letters to his friend Mme. Raffalovich: "The scientist must have imagination, but he must master this imagination and coldly probe the unknown. However, if he lets himself be carried away by his imagination, he will be overcome by vertigo and, like Faust and others, fall into the chasm of magic and succumb to phantoms of the mind." Early in his career, he aimed to follow the fate of the sugar absorbed from the food in the animal body. The hypothesis at that time was that the ingested sugar was burned in the lungs, passing the liver through the hepatic portal system (see Box 8.1) without any processing to reach the bloodstream. To confirm this hypothesis (and also to discard that the sugar was not destroyed in the liver, as it seems that he suspected), Claude Bernard measured the amount of sugar in the portal and hepatic veins of a dog fed with sweet milk. He would be convinced when he found a large amount of glucose in the blood that had passed through the liver, but he was not, as he pointed out on his book *Introduction à l´Étude de la Medicine Expérimentale*: "More than one researcher would have stopped here and would have thought that any control experiment was useless. But I performed a control experiment because I am convinced that in physiology

(continued)

Box 9.4 (continued)
you should always doubt even if the doubt doesn't seem to be permitted." The control was a similar experiment in which the dog was fed only with meat. For his surprise, he found a large amount of sugar in the hepatic vein, which led him to state: "I don't understand anything anymore."


Claude Bernard (1813-1878)

This unexpected finding led Claude Bernard to isolate glycogen some years later, but his contribution to the understanding of animal metabolism did not stop there. The observation that liver releases glucose into the blood led him to establish the concept of "internal secretion." Additionally, Claude Bernard was the first to express the idea that the animals have an inner milieu, different from the external environment, whose constancy would be a requirement for life maintenance (the basis from which the principle of homeostasis emerged), as he stated: "la fixité du milieu intérieur est la condition d'une vie libre et indépendent" (the stability of the internal environment is the condition for a free and independent life).

molecules may be converted to glucose by a pathway named gluconeogenesis. This will be discussed in the next sections of this chapter.

In many tissues, especially in the liver and muscles, glycogen is the storage form of glucose, being observed as dense granules at the microscope (see Fig. 8.5). Liver glycogen content may correspond to about 10 % of wet weight of this organ in well-fed humans. In the muscles, glycogen content can account for 1–2 % of their wet

weight, but since the muscles occupy a much larger area of the body than the liver, total muscle glycogen content is twice as high as that of liver.

The process of glycogen degradation, known as glycogenolysis, was almost entirely elucidated by Carl and Gerty Cori. They discovered (a) the nature of glycogen degradation reaction, a phosphorolysis reaction; (b) the enzyme that catalyzes it, glycogen phosphorylase; and (c) its product, glucose-1-phosphate. Due to this great contribution to carbohydrate metabolism, Carl and Gerty Cori were awarded the Nobel Prize in Physiology or Medicine in 1947.

Carl Ferdinand Cori (1896-1984)
Gerty Theresa Cori (1896-1957)

9.2.1 Reactions of Glycogen Degradation

Glycogen is a highly branched polymer of glucose containing α1,6-glycoside linkages in the branch points, with 10–12 glucose residues linked by α1,4-glycoside linkages between each branch (see Sect. 3.2.1). Its degradation depends on two enzymes, the glycogen phosphorylase (GP) and the debranching enzyme.

GP catalyzes the phosphorolysis of the α1,4 glycoside linkage at a terminal glucose unit from the nonreducing ends of the molecule, yielding glucose-1-phosphate (Fig. 9.3). In their first studies on glycogen degradation, the Coris showed that the reaction catalyzed by GP was reversible in vitro. However, now it is clear that phosphorolysis is greatly favored in vivo due to the very high intracellular ratio [Pi]/[glucose-1-phosphate], and glycogen synthesis has to occur through another pathway (see Sect. 8.2).

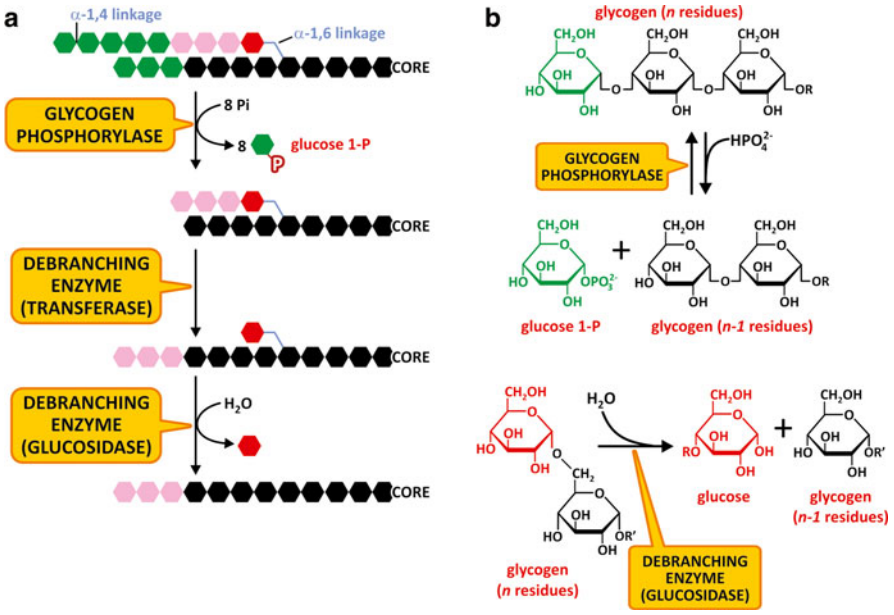


Fig. 9.3 (**a**) Schematic representation of glycogen degradation. (**b**) Reactions catalyzed by glycogen phosphorylase (*top*) and the glucosidase activity of the debranching enzyme (*bottom*)

The branched structure of glycogen enables its rapid degradation, since about 50 % of glucose units are in the outer branches. Glucose units are removed sequentially until four units before the branch point, where the enzyme loses its activity leaving what is known as a limit dextrin (a molecule with short branches of four glucose units in length).

Further removal of the glucose units from a glycogen molecule depends on the activity of the debranching enzyme. This enzyme has two different activities in the same polypeptide chain. The first is a glucosyltransferase activity, in which a trisaccharide unit at the end of a branch is transferred through an α1,4-linkage to a nonreducing end of another chain, resulting in an extended chain susceptible to GP action (Fig. 9.3). The second activity is an amylo-1,6-glucosidase, in which the α1,6-glycoside linkage in the branch points is hydrolyzed to form glucose. It is interesting to note that due to this activity, about 7 % of the glucose residues in glycogen are released as glucose and not as glucose-1-phosphate.

Glucose-1-phosphate produced during glycogenolysis may be converted to glucose-6-phosphate by the action of the enzyme phosphoglucomutase (Fig. 9.3).

In the liver, glucose-6-phosphate may be dephosphorylated through the action of glucose-6-phosphatase (G6Pase), allowing glucose units to be removed from glyco-

gen and released in the bloodstream. G6Pase is an integral protein of the endoplasmic reticulum membrane with the active site on the luminal side of this compartment (Fig. 9.4). It is expressed only in the liver and kidney, and because muscle cells lack this enzyme, the glucose units removed from muscular glycogen are metabolized in muscle tissue itself.

In their first physiological studies on carbohydrate metabolism, the Coris proposed that the lactate produced in muscle from glycogen degradation (and subsequent glycolysis) reached the liver through the bloodstream being then reconverted to glycogen, in a cycle that became known as the "Cori cycle." However, although the idea of cycling metabolites between tissues is a very important concept, the "Cori cycle," exactly as it was proposed, does not occur physiologically. Glucose produced in the liver from muscle lactate, instead of being converted to glycogen, is released into the bloodstream to be used by the brain, as well as by erythrocytes and other fermentation-dependent cells. Additionally, it is very unlikely that glucose produced in the liver goes to the muscles, since its internalization in muscle cells depends on the insulin action, which is not operating in this situation (see Sect. 8.4). In fact, we can construct a more complex picture of glycogen/glucose-lactate cycling in different organs, as presented in Fig. 9.5.

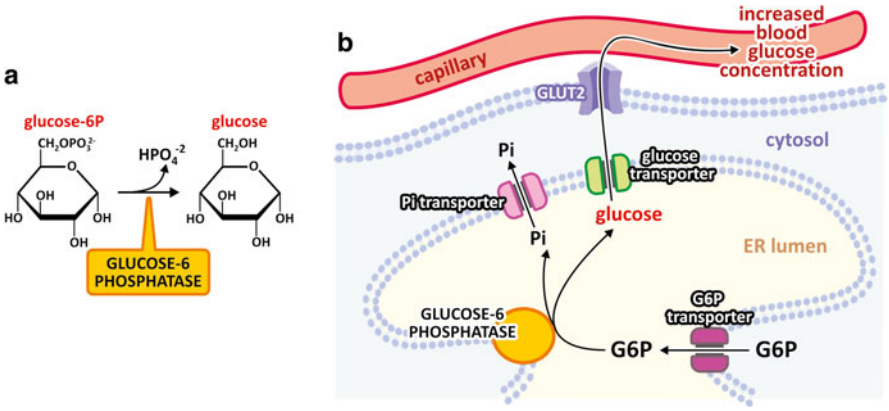


Fig. 9.4 (**a**) Glucose-6-phosphatase reaction. (**b**) Intracellular localization of glucose-6-phosphatase. The enzyme is an integral protein in the endoplasmic reticulum (ER) membrane that catalyzes the dephosphorylation of glucose-6-phosphate in the lumen of this organelle. The enzyme substrate (glucose-6-phosphate) and products (glucose and inorganic phosphate) are transported across ER membrane through specific transporters

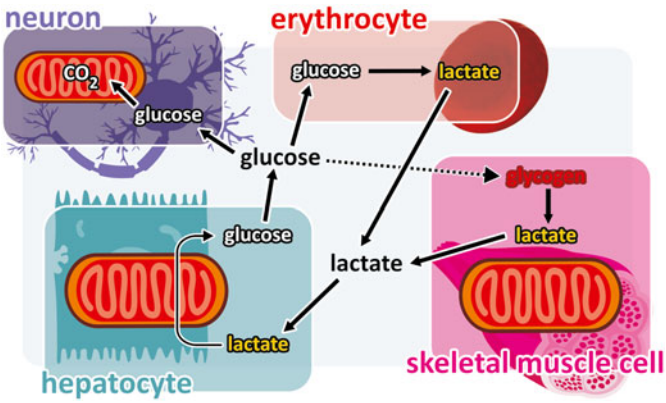


Fig. 9.5 Glycogen/glucose-lactate cycling: glycogen degradation in the muscles forms lactate, which is released in the bloodstream and enters the liver, where it is converted to glucose by gluconeogenesis. Glucose released from the liver into the bloodstream is taken up by the glucose-dependent cells, such as those from the brain and the erythrocytes. Uptake of glucose by the skeletal muscle (forming the so-called Cori cycle) is hypothetical because the physiological conditions that favor lactate production by muscle cells do not favor glucose uptake by these cells (see text)

9.2.2 Regulation of Glycogen Degradation in the Liver

Liver glycogen content largely varies in response to food intake (Fig. 9.6). Glycogen accumulates rapidly after carbohydrate ingestion and then it is gradually mobilized to generate free glucose in between the meals.

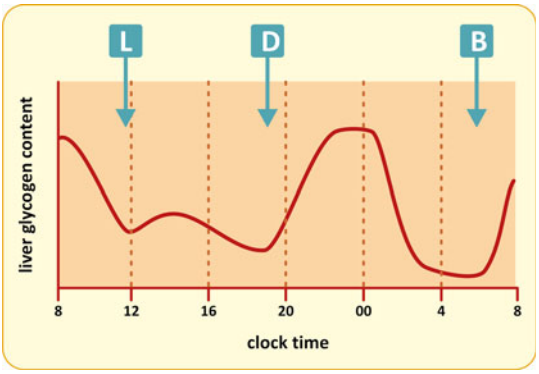


Fig. 9.6 Variation in the liver glycogen content after each meal

Glycogen degradation is mainly controlled by regulating GP activity. This enzyme is a dimer that exists in two different conformations, one more active, named phosphorylase *a*, and one much less active, named phosphorylase *b* (Fig. 9.7). These two forms are interconvertible by phosphorylation/dephosphorylation of a serine residue (Ser14), induced by hormone action (see Sect. 9.3.3).

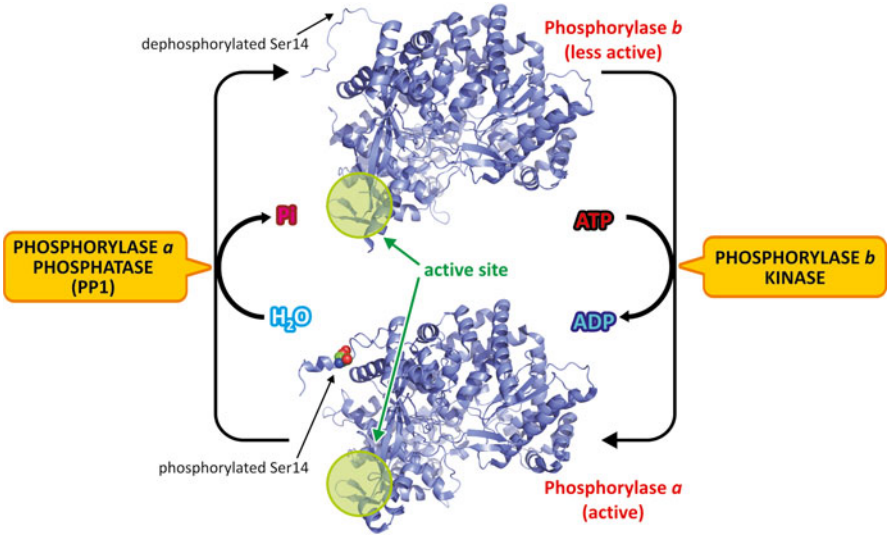


Fig. 9.7 Regulation of GP activity by phosphorylation. In the liver, glucagon action triggers the phosphorylation of the less active form of GP, named phosphorylase *b* (PDB 1FC0), in its Ser14, which promotes a structural change to the active form, phosphorylase *a* (PDB 1FA9). The N-terminal segment, which contains the phosphorylation site (Ser14), converts from a completely disordered conformation to a well-ordered structure, and several structural transitions occur in the active site (highlighted in *green*). To facilitate the visualization of enzyme conformational changes, the monomer structures are shown in the figure, although the enzyme exists as homodimers

The major allosteric modulator of the liver isoform of GP is glucose, which shifts the equilibrium between the conformational states to phosphorylase *b* (Fig. 9.7). Thus, when the intracellular concentration of glucose is high, glycogen degradation is inhibited. The allosteric regulation of the muscle isoform of GP is more complex and will be discussed in Chap. 7.

Glycogen degradation is also controlled by hormones. In a situation of hypoglycemia, insulin secretion by the pancreatic β-cells is inhibited, leading to an increase in glucagon secretion by the α-cells. The action of glucagon on liver tissue (detailed in Sect. 9.4) results in the activation of the enzyme phosphorylase kinase, which catalyzes the phosphorylation of GP. Phosphorylation maintains GP in the active form (Fig. 9.7), favoring glycogen degradation. The hormone adrenaline also controls glycogen degradation in the liver (discussed in the exercise situation in Chap. 10).

It is important to note that in humans glycogen stored in the liver lasts between 12 and 24 h during fasting (see Fig. 9.2). Therefore, the contribution of liver glycogenolysis to the control of glycemia is limited and another glucose-producing pathway is required to maintain blood glucose concentration.

9.3 Gluconeogenesis

Gluconeogenesis is the synthesis of glucose (and other carbohydrates) from non-glycidic compounds. This pathway occurs mainly in the liver, but also in the kidney cortex.

For many years it was thought that gluconeogenesis occurred as a reversal of the glycolytic pathway. However, some of the glycolysis reactions are highly exergonic (Fig. 9.8), which makes it very unlike that they could be reversed within the cells.

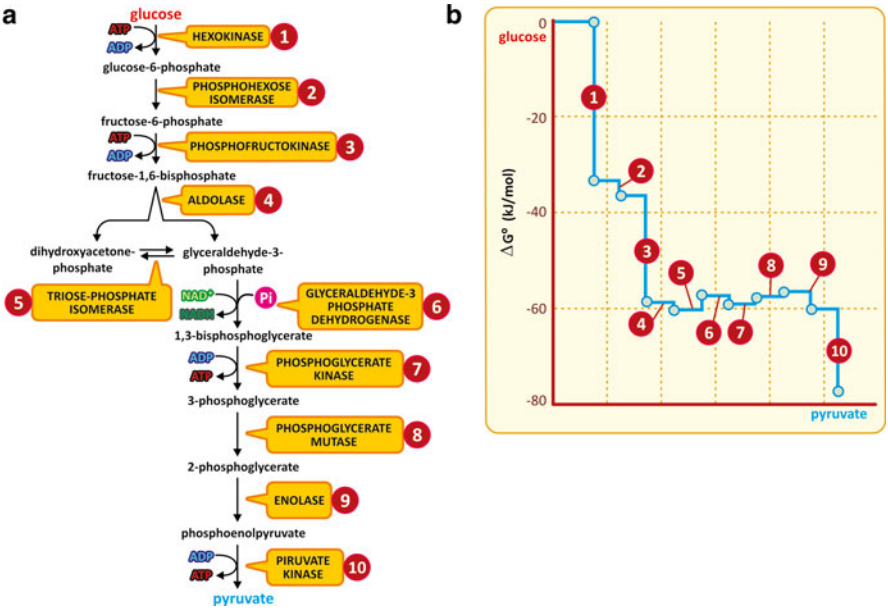


Fig. 9.8 (**a**) Glycolysis reactions (for more details, see Chap. 6). (**b**) Free energy variation between each step of glycolysis. The numbers correspond to the reactions indicated in (**a**)

This energy barrier impairs reversibility at three points of the glycolytic pathway: the conversion of (a) pyruvate in phosphoenolpyruvate (PEP), (b) fructose-1,6-bisphosphate in fructose-6-phosphate, and (c) glucose-6-phosphate in glucose. For these reactions to occur, glycolytic enzymes should be bypassed.

9.3.1 Gluconeogenesis Reactions

The first bypass is the conversion of pyruvate in PEP, which requires two reactions involving two enzymes: pyruvate carboxylase (PC) and PEP carboxykinase (PEPCK).

PC is a mitochondrial enzyme that catalyzes the biotin-dependent carboxylation of pyruvate to produce oxaloacetate. Human PC is active in the tetrameric form and contains three functional domains in the same polypeptide chain: biotin-carboxyl carrier protein (BCCP), biotin carboxylase (BC), and carboxyltransferase (CT) domains (Fig. 9.9; see also Box 8.6 for more information on biotin-dependent carboxylases).

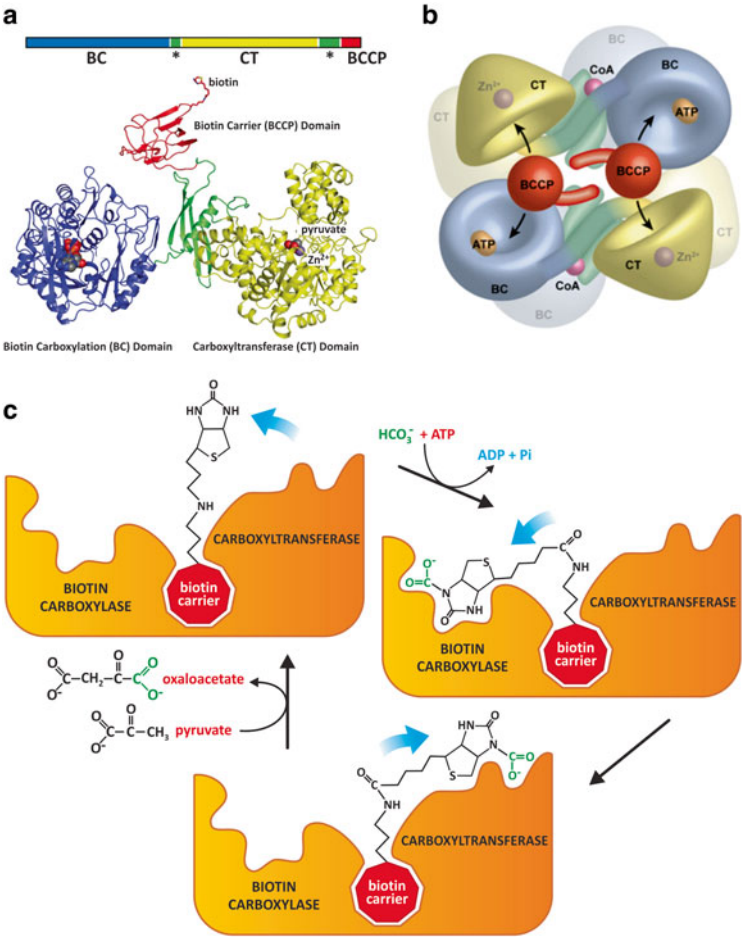


Fig. 9.9 (**a**) PC primary structure and the structure of the *Staphylococcus aureus* PC monomer highlighting the three functional domains: BC (*blue*), CT (*yellow*), and BCCT (*red*), and the allosteric domain (*green*). (**b**) Schematic representation of PC tetramer showing the movement of BCCP domain between neighboring BC and CT active sites in opposite polypeptide chains (Reproduced from Jitrapakdee et al. Biochem. J. 413:369–387, 2008. Portland Press Ltd, London). (**c**) Reactions catalyzed by PC: first bicarbonate is used to carboxylate the enzyme-linked biotin in an ATP hydrolysis-dependent reaction; then the carboxyl group is transferred to pyruvate generating oxaloacetate

Additionally, there is a fourth central structural domain that contains the binding site of the allosteric activator acetyl-CoA. Biotin is covalently linked to a Lys residue in the BCCP domain. The reaction occurs in two steps. First, the BC domain catalyzes the carboxylation of biotin in a reaction that uses bicarbonate as a substrate and requires the hydrolysis of one ATP molecule, generating ADP and Pi (Fig. 9.9). Then, the carboxyl group from carboxybiotin is transferred to pyruvate to form oxaloacetate in a reaction catalyzed by the CT domain of the enzyme. In PC tetrameric form, the domains are arranged in such a way that carboxybiotin is transferred from BC domain to the neighboring CT domain on opposing polypeptide chains, explaining why the enzyme is only active as a tetramer (Fig. 9.9).

Biotin-dependent carboxylation occurs in other metabolic pathways, such as the synthesis of fatty acids (see Sect. 8.3), in which acetyl-CoA carboxylase BC domain shares sequence homology with the BC domain of PC.

It is important to point out that besides its crucial role in gluconeogenesis, the PC reaction plays an important anaplerotic function, replenishing oxaloacetate that is withdrawn from TCA cycle in many metabolic situations (see Sect. 7.3).

The formation of PEP from oxaloacetate is catalyzed by PEP carboxykinase (PEPCK), in a reaction that requires the transfer of a phosphoryl group from GTP (Fig. 9.10).

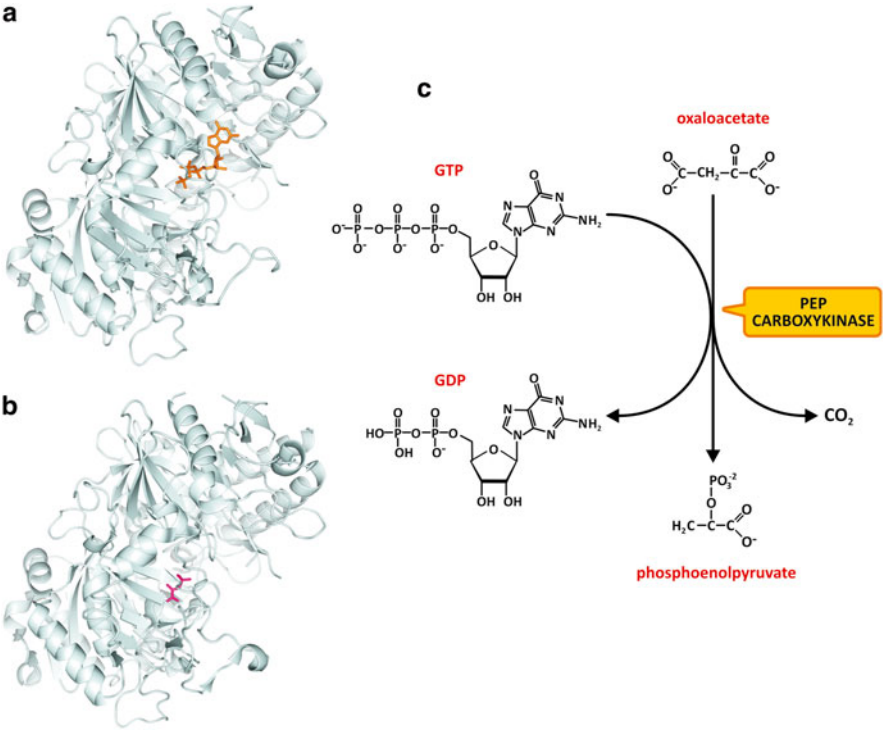


Fig. 9.10 Structure of human cytosolic PEPCK: (**a**) complexed with a non-hydrolysable GTP analogue (PDB 1KHE) and (**b**) complexed with PEP (PDB 1KHF). (**c**) Conversion of oxaloacetate in PEP, using GTP as the phosphate donor, the reaction catalyzed by PEPCK from animals. Differently, bacterial, fungal, and plant PEPCKs use ATP as the phosphate donor

In human cells, PEPCK is distributed between mitochondria and the cytosol, and the isoform used will depend indirectly on the [NADH]/[NAD⁺] ratio in the cytosol. The reason for this is that the reaction catalyzed by glyceraldehyde-3-phosphate dehydrogenase requires NADH to occur in the reverse direction of glycolysis, from 3-phosphoglycerate to glyceraldehyde-3-phosphate (Fig. 9.11).

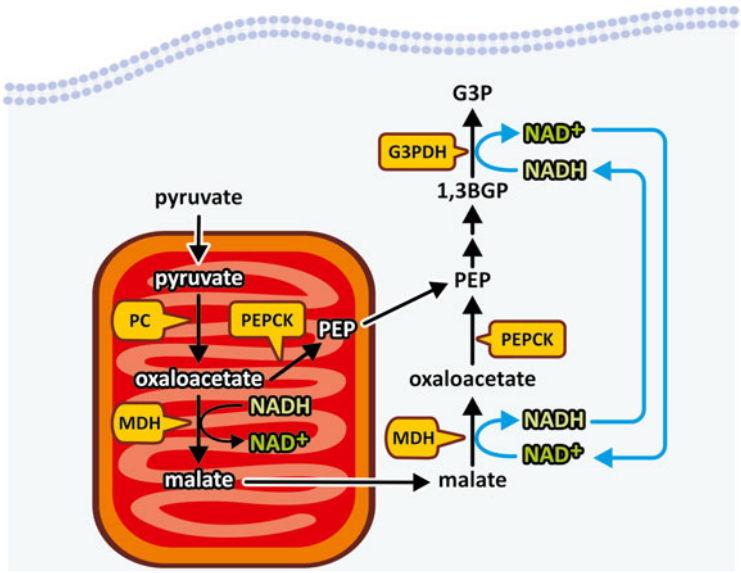


Fig. 9.11 Intracellular location of the first gluconeogenesis bypass. PC is a mitochondrial enzyme, and thus pyruvate is converted to oxaloacetate inside the mitochondria. PEPCK is distributed equally in cytosol and mitochondria, so oxaloacetate may be converted to PEP in both cellular compartments. When cytosolic [NADH]/[NAD⁺] ratio allows the reaction catalyzed by glyceraldehyde-3-phosphate dehydrogenase (G3PDH) to occur from 1,3-bisphosphoglycerate (1,3-BPG) to glyceraldehyde-3-phosphate (G3P), the mitochondrial isoform may be used. When the formation of additional NADH is required, oxaloacetate is converted to malate in the mitochondrial matrix by malate dehydrogenase (MDH) with NADH oxidation. Malate is transported to the cytoplasm where it is converted to oxaloacetate by the cytosolic MDH, generating NADH for the G3PDH reaction

When [NADH]/[NAD⁺] ratio is very low, NADH equivalents are transported from the mitochondrial matrix to the cytosol by the malate–oxaloacetate shuttle. Oxaloacetate is converted to malate by the mitochondrial malate dehydrogenase, with NADH oxidation to NAD⁺ into the mitochondria. Malate crosses the mitochondrial membranes and reaches the cytosol where it is reconverted into oxaloacetate through the action of the cytosolic malate dehydrogenase, with NADH formation in the cytosol.

Therefore, in this situation, cytosolic PEPCK is used to generate PEP, while mitochondrial PEPCK is preferred when cytosolic [NADH]/[NAD^+] ratio allows glyceraldehyde dehydrogenase reaction to occur in the direction of glyceraldehyde-3-phosphate formation (Fig. 9.11).

The second bypass is the conversion of fructose-1,6-bisphosphate to fructose-6-phosphate. In this reaction, the phosphoryl group associated to carbon 1 of fructose-1,6-bisphosphate is removed by hydrolysis through the action of the enzyme fructose-1,6-bisphosphatase.

Finally, the third bypass is the conversion of glucose-6-phosphate to glucose. As discussed in the previous section, in the liver as well as in the kidneys, the enzyme glucose-6-phosphatase catalyzes the dephosphorylation of glucose-6-phosphate generating glucose, which can be released into the bloodstream (see Fig. 9.4).

9.3.2 Precursors for the Synthesis of Glucose

At this point it is already possible to visualize how different precursors can enter the gluconeogenesis pathway. As detailed below, the main precursors are lactate, amino acids, and glycerol. Additionally, propionyl-CoA formed in the oxidation of odd-chain fatty acids can also contribute to glucose synthesis, although in a much lower scale due to the low availability of odd-chain fatty acids in human metabolism.

Several studies in the beginning of the twentieth century showed that lactate could be converted to glucose (and glycogen) in liver tissue. At that time, the interest was focused on the synthesis of glycogen in the body and less attention was given to the reactions involved in the conversion of lactate to glucose. Now we know that lactate conversion to glucose (gluconeogenesis) and glucose conversion to glycogen (glycogenogenesis) are processes that do not occur simultaneously (see the last section of this chapter). Probably as a result of this, there were many controversial observations at that time.

Lactate enters the gluconeogenesis pathway through its conversion to pyruvate in a reaction catalyzed by the enzyme lactate dehydrogenase (Fig. 9.12). In this reaction, cytosolic NAD^+ is reduced to NADH, increasing [NADH]/[NAD^+] ratio in the cytosol and favoring PEP formation inside the mitochondria (see previous section).

Evidence that amino acids can act as substrates for glucose synthesis came from the early studies on glycogen formation by Claude Bernard in the nineteenth century, in which he fed dogs with meals consisting solely of meat and found an increase of glucose released by the liver (see Box 9.4).

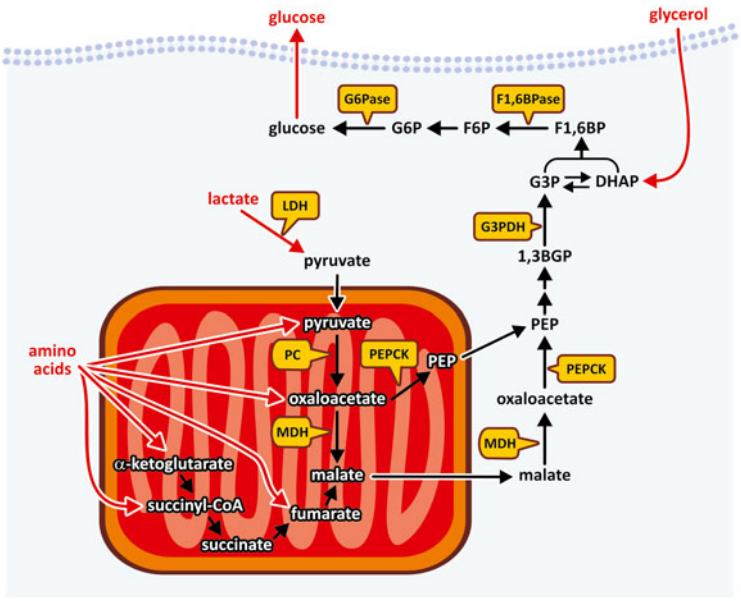


Fig. 9.12 Entry of precursors in the gluconeogenesis pathway. Lactate is converted to pyruvate by lactate dehydrogenase (LDH). Amino acids have their amino groups removed by transamination and/or deamination generating TCA cycle intermediates that converge to malate, which is converted to oxaloacetate and then to PEP in the cytosol. Glycerol is converted to glycerol phosphate and then to dihydroxyacetone phosphate (DHAP), entering gluconeogenesis. F1,6BP, fructose-1,6-bisphosphate; F1,6BPase, fructose-1,6-bisphosphatase; F6P, fructose-6-phosphate; G6P, glucose-6-phosphate; G6Pase, glucose-6-phosphatase; other abbreviations are the same as in Fig. 9.11. The names of the enzymes are highlighted in *yellow boxes*

To enter gluconeogenesis, the amino group of the amino acids should be removed by transamination and/or deamination. After this metabolization, eighteen from the twenty more common amino acids generate α-ketoglutarate, succinyl-CoA, fumarate, oxaloacetate, or pyruvate (see Sect. 7.5). The TCA cycle intermediates converge to malate through the action of the enzymes of the cycle. Malate leaves mitochondria and is converted to oxaloacetate, which then forms PEP in the cytosol (Fig. 9.12).

The product of the metabolization of leucine or lysine is acetyl-CoA, which cannot be used to synthesize glucose since its two carbon atoms are completely oxidized to CO_2 in TCA cycle, thus impairing the net accumulation of carbons to be incorporated in the newly synthesized glucose molecule. This is also the reason why fatty acids cannot be transformed in glucose in animals.

Finally, glycerol, which is converted to glycerol phosphate after being transported to the cells, is converted to dihydroxyacetone phosphate by glycerol-phosphate dehydrogenase, entering gluconeogenesis at this point (Fig. 9.12).

9.3.3 *Regulation of Gluconeogenesis*

Glycolysis and gluconeogenesis share many reactions and both pathways are exergonic in the intracellular conditions. Thus, these pathways must be reciprocally regulated to allow one of them to predominate over the other in each specific situation. As a matter of fact, in the liver, the rates of glycolysis and gluconeogenesis are adjusted to maintain the blood glucose concentration stable.

The main point of the reciprocal regulation of glycolysis and gluconeogenesis is the interconversion between fructose-6-phosphate and fructose-1,6-bisphosphate by the enzymes phosphofructokinase-1 (PFK-1) in glycolysis and fructose-1,6-bisphosphatase (F1,6BPase) in gluconeogenesis. This point is controlled by hormonal action with a key role of the molecule fructose-2,6-bisphosphate (Fig. 9.13).

Fructose-2,6-bisphosphate in submicromolar concentrations simultaneously activates PFK-1 and inhibits F1,6BPase. This molecule is synthesized through the phosphorylation of fructose-6-phosphate in a reaction similar to that catalyzed by PFK-1, but with the transfer of the phosphoryl group of ATP to the carbon 2 instead of the carbon 1 of fructose. The enzyme that catalyzes this reaction was named phosphofructokinase-2 (PFK-2) to avoid confusion with the classic PFK-1. Fructose-2,6-bisphosphate is hydrolyzed to fructose-6-phosphate by the enzyme fructose-2,6-bisphosphatase (F2,6BPase).

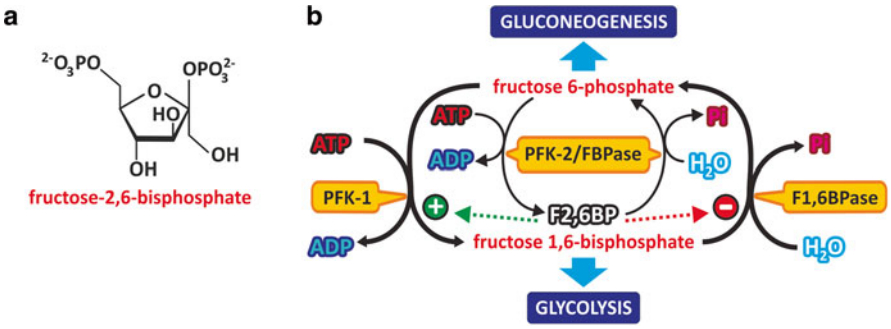
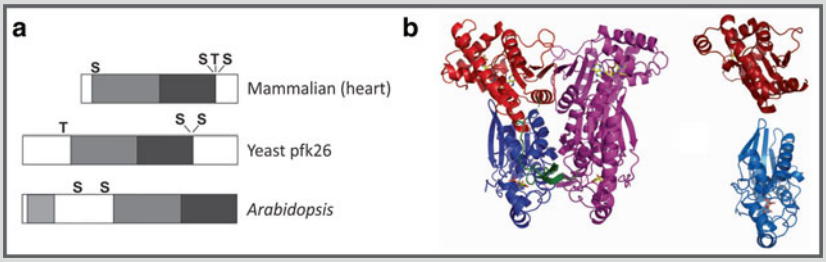


Fig. 9.13 (**a**) Fructose-2,6-bisphosphate structure. (**b**) Synthesis and degradation of fructose-2,6-bisphosphate and its effect on the activities of PFK-1 and F1,6BPase in the liver

It is very interesting to note that these two antagonic activities, PFK-2 and F2,6BPase, are present in the same polypeptide chain, an example of a bifunctional enzyme (Box 9.5).

Box 9.5: Evolution of the Bifunctional Enzyme PFK-2/F2,6BPase

It is believed that the bifunctional enzyme PFK-2/F2,6BPase resulted from the fusion of genes encoding different enzymes. This seems to have occurred very early during evolution in a common ancestor of all eukaryotes. In all taxonomic groups, the enzyme has a central catalytic core with the PFK-2 and F2,6BPase domains in tandem and extensions in N- and C-terminals [see examples in (**a**)]. In some cases, one of the activities is damaged by deletions or insertions. The kinase and the phosphatase domains are structurally similar to one-domain kinases or phosphatases (see figure).



(**a**) Schematic representation of domain localization of PFK-2 and F2,6BPase primary sequence in different organisms. (**b**) Comparison of the structure dimeric human bifunctional enzyme with gluconate kinase, in *red*, and PhoE phosphatase, in *blue*. Reproduced with permission from Michels & Rigden. IUBMB Life 58:133–141, 2006

The bifunctional enzyme presents a regulatory domain in its N-terminal end, which contains a serine residue (Ser32) that can be phosphorylated or dephosphorylated in response to the action of glucagon (see Sect. 9.4.1) or insulin (see Sect. 8.4), respectively. When Ser32 is phosphorylated, the enzyme undergoes a conformational change that favors the F2,6BPase activity. In contrast, when it is dephosphorylated, the enzyme activity is turned to PFK-2.

Gluconeogenesis is also regulated at the first bypass level. Carboxylation of pyruvate to oxaloacetate is completely dependent on the presence of acetyl-CoA, which acts as a specific activator of PC by its reversible binding to the allosteric domain of the enzyme (see PC structure in Fig. 9.9).

This is an interesting example of how the information of a physiological situation can be transmitted locally to a specific cellular compartment by means of an allosteric modulator. In hypoglycemia, triacylglycerides stored in the adipose tissue are mobilized, generating glycerol and fatty acids (see Sect. 7.4.1). The increased availability of fatty acids in the bloodstream allows them to be used as energy source for many tissues, including the liver. β-oxidation of fatty acids, which occurs within the mitochondria (see Sect. 7.4.4), increases the concentration of acetyl-CoA

in the mitochondrial matrix where PC is located. Thus, the mobilization of triglycerides as a response to hypoglycemia results in the generation of an essential activator of an important enzyme for the synthesis of glucose, a crucial pathway in this situation.

The activity of PEPCK is regulated only at the transcriptional level. The gene that encodes the cytosolic form of PEPCK in the liver contains several hormone response elements, including glucocorticoid, cyclic AMP, and insulin response units, besides thyroid hormones and retinoic acid response units. Through different mechanisms of actions, glucocorticoids and glucagon enhance the transcription of PEPCK gene (see Sect. 9.4), while insulin represses its basal and hormone-induced expression.

9.3.4 Dynamic Utilization of Gluconeogenesis Precursors

Daily glucose requirement for the adult human organism is around 120 g. In a fasting situation, in which no glucose is ingested, the contribution of each of the gluconeogenesis precursors is shown in Table 9.2.

Table 9.2 Contribution of each gluconeogenesis precursors to glucose production

	Amount of glucose produced daily (g)	
Precursor	1-day starvation	5 weeks' starvation
Lactate	39	39
Glycerol	19	19
Amino acids	60	16

Taking into account that each precursor is originated in a specific tissue, the blood concentration of glucose can be maintained due to the interplay among different tissues of the organism (Fig. 9.14). Glycerol is generated by the hydrolysis of triacylglycerols in the adipose tissue. Proteolysis of the contractile proteins in muscle cells generates amino acids that undergo transamination, mainly with pyruvate or α-ketoglutarate within muscle cells, forming alanine and glutamine. Lactate is continuously produced by the fermentation-dependent cells such as the erythrocytes.

It is important to note that this situation changes if glucose is not ingested for longer periods. If protein mobilization was maintained at the same rate as in the beginning of fasting, after about one month without food ingestion, half of the body proteins would be consumed. This can be calculated from the data shown in Tables 9.1 and 9.2 (for these calculations, the stoichiometry of glucose synthesis from amino acids must be taken into account: to produce one gram of glucose, about two grams of amino acids are necessary).

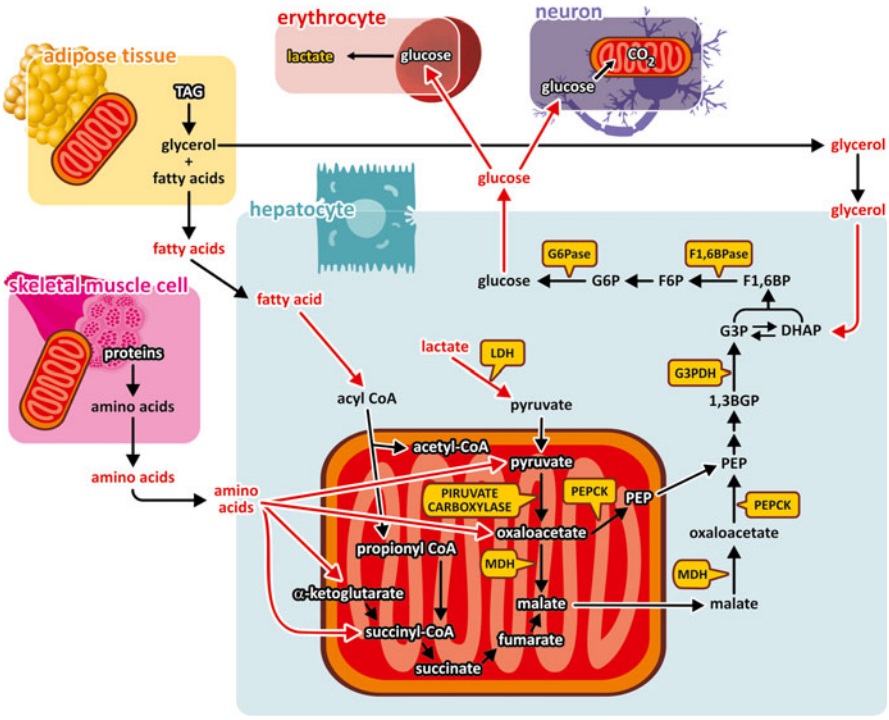
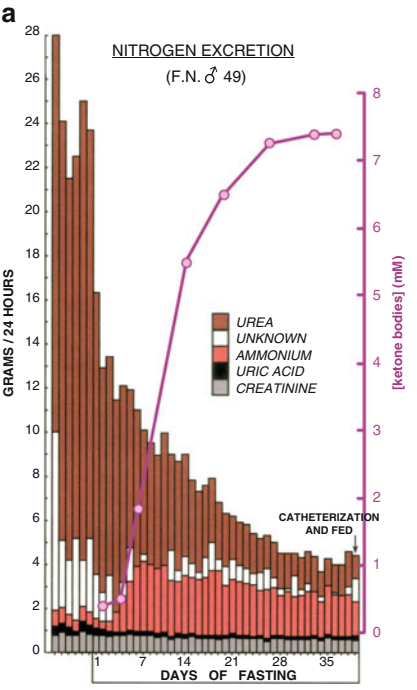


Fig. 9.14 Metabolite interplay among the organs. Hydrolysis of triacylglycerols in the adipose tissue generates glycerol and fatty acids that are released in the bloodstream. Even-chain fatty acids form acetyl-CoA, while each molecule of odd-chain fatty acid forms one molecule of propionyl-CoA besides acetyl-CoA. Propionyl-CoA may enter gluconeogenesis after its conversion to succinyl-CoA and then malate through TCA cycle reactions. Glycerol enters the liver cells where it is converted to DHAP and then to glucose. Proteolysis of the contractile proteins in muscle cells generates amino acids that are released in the bloodstream entering the liver, where they undergo transamination/deamination generating pyruvate or TCA cycle intermediates that ultimately form malate to enter the gluconeogenesis pathway. Lactate is continuously produced by and released from the fermentative cells such as the erythrocytes, reaching the liver where it is converted to pyruvate, which enters the gluconeogenesis pathway. Glucose produced is mainly used by the cells from central nervous systems and by the fermentation-dependent cells. Abbreviations are the same as used in Fig. 9.12

However, the human organism is adapted to survive much longer periods of starvation. Even in the therapeutic starvation for obesity treatment as presented in the beginning of this chapter, the patients spent more than one month without any food ingestion. The studies performed with these patients made possible to understand this adaptation. Catheterization of brain vessels demonstrated that two thirds of brain fuel consumption in long starvation corresponded to the metabolization of β-hydroxybutyrate and acetoacetate, markedly diminishing the need of glucose production and, consequently, muscle proteolysis to provide gluconeogenic precursors. In fact, the decrease in protein degradation during fasting can be inferred

by observing the profile of nitrogen excretion of a patient submitted to therapeutic starvation. It is interesting to note that the decrease in nitrogen excretion shows a clear correlation with the increase in ketone bodies' concentration in the blood (Fig. 9.15a).



b

Species	Brain weight (g)	Brain/body weight (%)
Australopitecus africanus	445	1.0
Homo habilis	650	1.5
Homo erectus	940	1.9
Homo neanderthensis	1420	1.9
Homo sapiens		
Adult	1400	2.3
Newborn	400	11.4
Pantroglodytes (chimpanzee)		
Adult	400	0.9
Newborn	150	10

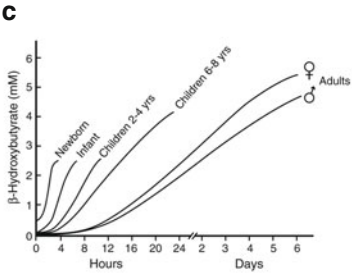


Fig. 9.15 (**a**) Excretion of different nitrogenated compounds by a human subject during starvation (Reproduced from Owen, Biochem. Mol. Biol. Educ. 33:246–251, 2005) and its correlation to the concentration of ketone bodies (β-hydroxybutyrate and acetoacetate) in the blood. (**b**) Encephalization during human evolution (Reproduced from Cunnane & Crawford, Comp. Biochem. Physiol. 136:17–26, 2003, with permission from Elsevier). (**c**) Age dependence of β-hydroxybutyrate concentration in blood during fasting (Reproduced with permission from: Cahill. Ann. Rev. Nutr. 26:1–22, 2006)

To better understand this adaptation, it is important to have some information regarding the brain uptake of ketone bodies. Ketone bodies are transported across the blood–brain barrier through the monocarboxylate transporters (MCTs), especially MCT1, which is highly expressed in endothelial cells that form blood–brain barrier vessels. MCT1 transports a wide range of short-chain monocarboxylates, including lactate, pyruvate, acetoacetate, and β-hydroxybutyrate. The K_M values for these substrates are in the range of 5–10 mM. Thus, the increase in ketone bodies concentration in the blood during fasting greatly favors the transport of these molecules across the blood–brain barrier. As seen in Fig. 9.15a, blood concentration of

β-hydroxybutyrate reaches the range of the K_M for MCT1 after the first days of fasting, in such a way that this molecule becomes increasingly available to brain cells as fasting proceeds. Additionally, prolonged ketonemia, as it occurs in starvation or low-carbohydrate diets, induces MCT1 gene expression, also contributing to the increase of MCT1-mediated ketone bodies' transport to the cells of the central nervous system. Thus, along the first week of fasting, the use of ketone bodies by central nervous system cells greatly decreases the requirement of glucose as the energy supply for these cells. Since brain metabolism accounts for most of the use of glucose in the body (approximately 100 g of the 120 g necessary daily), it is easy to imagine that gluconeogenesis rate can be considerably reduced as the blood concentration of ketone bodies increases.

The use of ketone bodies by the brain seems also to have been very important during human evolution, which was characterized by a remarkable increase of brain weight (Fig. 9.15b). However, the "cost" of encephalization is the increase in energy demands. The brain of a modern human adult corresponds to 2.3 % of its body weight but accounts for 23 % of energy consumption of the organism. In children, the energy demand for the brain is even greater (approximately 75 % of the total organism energy demand). The fact that the brain/body ratio is similar between humans and chimpanzees suggests that primates have in general the potential to have large brains. During development, however, the brain/body ratio in chimpanzees becomes less than a half of that of humans. An interesting observation that may explain this fact is that humans are the only among the primates that are born fat. This probably enables them to produce ketone bodies for brain use during the newborn development. As a matter of fact, human newborn metabolism is essentially ketotic, since the larger the brain/body ratio is, the more rapidly ketosis develops (Fig. 9.15c).

9.4 Hormonal Responses to Hypoglycemia

One of the major metabolic adaptations to hypoglycemia is the production of glucose by the liver. As discussed throughout the chapter, this is possible by the combination of hepatic glycogen degradation (in the first hours) and glucose production from non-glycidic precursors. Additionally, mobilization of triacylglycerol in the adipose tissue occurs simultaneously, ensuring the energy supplies for most of the tissues.

The simultaneous activation of these pathways is mainly regulated by the action of glucagon and glucocorticoids. The secretion of these hormones is enhanced by the decrease in blood glucose concentration and, through their action, the information about the hypoglycemia situation is transmitted to the different cells and organs.

In this section, the signaling pathways as well as the effects of glucagon and glucocorticoids on their main target cells will be detailed.

9.4.1 *Glucagon: Mechanism of Action and Effects on Energy Metabolism*

Glucagon is a peptidic hormone of 29 amino acid residues, secreted by the pancreatic α-cells, which compose the Langerhans islets together with β-cells that secrete insulin (see Sect. 8.4) and γ-cells that secrete somatostatin (Fig. 9.16).

Glucagon was discovered in the decade of the 1920s as a hyperglycemic factor produced by the pancreas. This finding was correlated to glycogen degradation in the liver, a subject extensively studied at that time (discussed in the beginning of this chapter), giving glucagon its original name of "hyperglycemic-glycogenolytic factor." During the twentieth century, the structure of the gene that encodes glucagon and the complex processing of its product as well as the glucagon signaling pathway and its effects on the target cells have been elucidated.

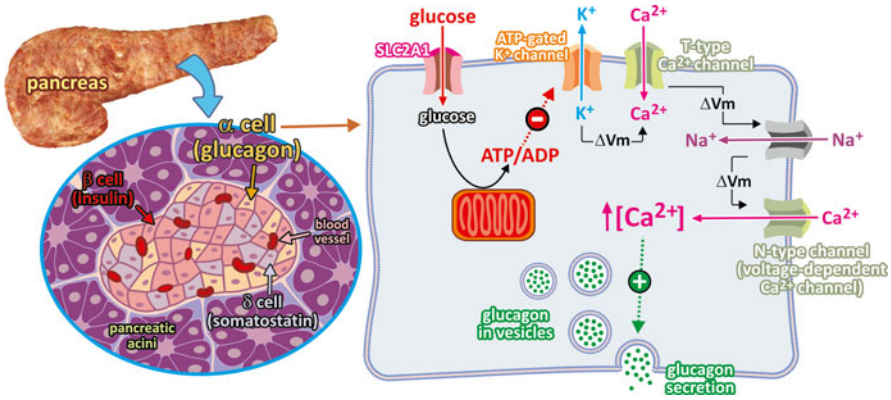


Fig. 9.16 Representation of a Langerhans islet, showing the α-cell and the mechanism of glucagon secretion in detail. Secretion of glucagon is controlled by a set of α-cell ion channels that generate action potentials of Na^+ and Ca^{2+}. At low levels of glucose, the activity of the ATP-sensitive K^+ channels renders a membrane potential that stimulates T-type Ca^{2+} channels to open, leading to membrane depolarization that, in turn, activates Na^+ and N-type Ca^{2+} channels. Intracellular Ca^{2+} waves caused by Ca^{2+} entry through N-type Ca^{2+} channels induce the exocytosis of glucagon granules. K^+ flow through A-type channels mediate membrane repolarization, so that this oscillatory electrical activity results in a pulsatile pattern of glucagon secretion. An increase in glucose consumption by α-cells rises the intracellular ATP/ADP ratio, blocking the ATP-sensitive K^+ channels. This causes membrane depolarization and decrease in Ca^{2+} influx, inhibiting glucagon secretion

At low levels of glucose, a set of ion channels on α-cell membrane generates action potentials that activate voltage-dependent L-type Ca^{2+} channels, leading to intracellular Ca^{2+} waves that induce the exocytosis of glucagon granules as well as

the expression of glucagon gene (Fig. 9.16). Membrane repolarization by K^+ flowing through A-type channels triggers oscillatory Ca^{2+} signals, so that glucagon secretion follows a pulsatile pattern. Although it seems clear that glucagon is released constitutively from α-cells, some factors, such as catecholamines and amino acids (mainly arginine), can act as positive regulators of its secretion by increasing the amplitude of the electric pulses in α-cells. However, the main control of blood concentration of glucagon occurs through the inhibition of its secretion. Glucose and insulin are the major negative regulators of glucagon secretion, so that the profile of blood glucagon concentration is the mirror image of that of glycemia. Therefore, glucagon transmits to the different organs in the body the information of hypoglycemia, leading to appropriate responses of specific tissues that allow the organism to deal with this situation.

Glucagon, like other hydrophilic hormones, acts on its target cells by binding to a receptor on the cell surface (see Chap. 5). Glucagon receptor is not uniformly distributed among the different tissues: it is expressed in high levels in the liver, kidneys, and pancreas and has also been detected in the adipose tissue and the heart, but it is absent in the skeletal muscle cells.

Glucagon receptor belongs to the superfamily of G protein-coupled receptors (see also Sect. 10.5.1.1). The main effects of glucagon on its target tissues are mediated by the increase of the intracellular levels of cyclic AMP (cAMP), through the classic mechanism of action of G protein-coupled receptors (Fig. 9.17). G proteins are composed of three subunits, α, β, and γ. α-Subunit binds GTP and catalyzes its hydrolysis, whose product, GDP, remains bound to the protein maintaining it in an inactive state, associated to β- and γ-subunits. When glucagon binds to its receptor, it undergoes a conformational change that is transmitted to G protein, leading to the replacement of GDP by GTP. When bound to GTP, the α-subunit dissociates from βγ-subunits and moves freely on the inner side of cellular membrane until it reaches the enzyme adenylate cyclase. This enzyme is activated by G protein α-subunit and catalyzes the conversion of ATP in cAMP, leading to an increase in the intracellular concentration of this molecule. The affinity of the α-subunit to adenylate cyclase decreases after GTP hydrolysis, leading the α-subunit itself to dissociate from adenylate cyclase and to reassociate to βγ-subunits.

The increase in the intracellular concentrations of cAMP as a response to glucagon binding to its receptor promotes the activation of an important enzyme, the cAMP-dependent protein kinase (PKA) (Fig. 9.17). This kinase is composed of two regulatory and two catalytic subunits. cAMP binds to the regulatory subunits, which dissociate from the catalytic ones that become free to catalyze the phosphorylation of several important enzymes, thus modulating their activities (as summarized in Fig. 9.17).

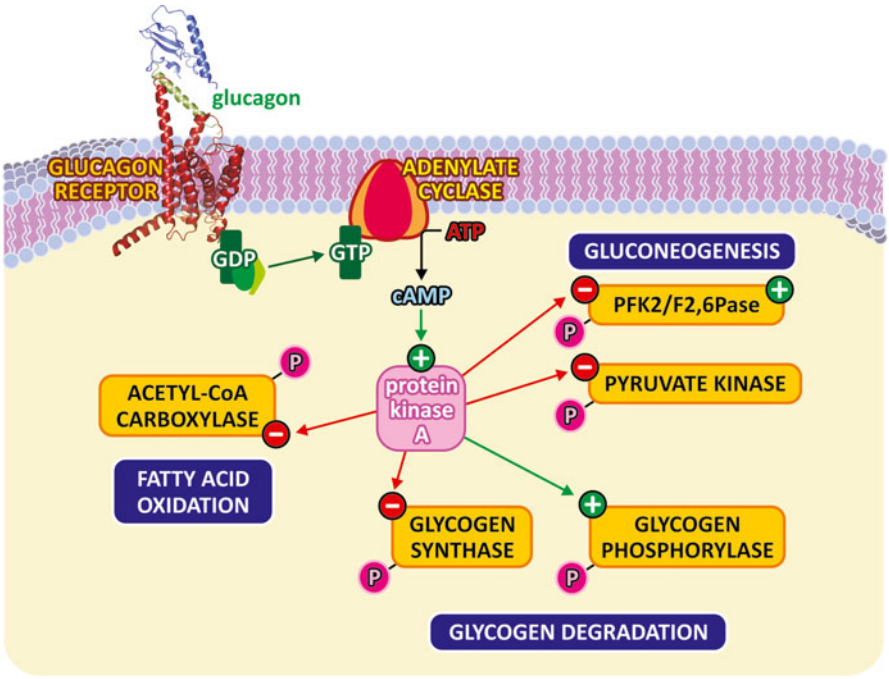


Fig. 9.17 Mechanism of glucagon action, showing its effects on enzyme activities in the liver. Glucagon (structure shown in green, PDB 1GCN) binds to its G protein-coupled receptor (PDB 4ERS and 4L6R), promoting the replacement of GDP by GTP in the G protein α-subunit, which dissociates from βγ-subunits and associates to adenylate cyclase, leading to an increase in the intracellular concentration of cAMP. This second messenger binds to the regulatory subunits (not represented in this simplified figure) of the protein kinase A (PKA), leading to the release of the active catalytic subunits. Active PKA catalyzes the phosphorylation of several enzymes (represented by the "P" in the *pink circle*), changing their activities as indicated by *green* "+," for activation, and the *red* "–," for inhibition. The pathways that are activated due to these changes in the enzyme activities are shown in the *blue boxes*

9.4.1.1 Effects of Glucagon on Liver Metabolism

Liver metabolism is drastically affected by glucagon. Hepatic glycogenolysis is activated, while glycogen synthesis is inhibited. Simultaneously, gluconeogenesis is activated and glycolysis inhibited. As a result, glucose is produced and released in the bloodstream. Additionally, fatty acid synthesis is inhibited (see Sect. 8.3), allowing the incoming fatty acids to undergo β-oxidation, ensuring ATP generation. The coordinated control of these different metabolic pathways is possible because key enzymes of each of these pathways have their activities modulated by phosphorylation promoted directly or indirectly by PKA (Fig. 9.18). Additionally, the increase in the intracellular concentration of cAMP can also regulate gene expression, since several genes present in their promoter region a cAMP-responsive element (CRE).

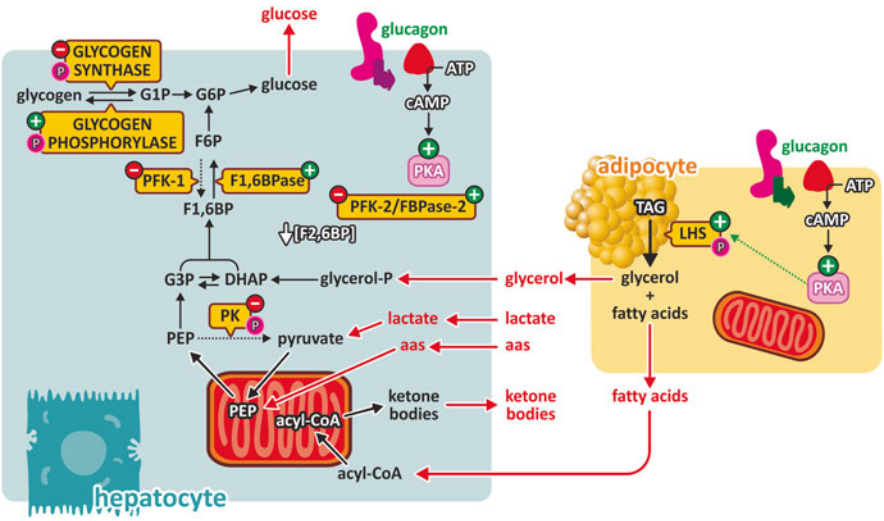


Fig. 9.18 Integration of the effects of glucagon on liver and adipose tissue, showing the enzymes and the metabolic pathways regulated in each cell. The P in the *pink circle* represents the phosphate group introduced in the enzyme by PKA; + and − indicate the activated or inhibited states, respectively. The *dashed lines* represent inhibited reactions. PFK-1, phosphofructokinase-1; F1,6BPase, fructose-1,6-bisphosphatase; PFK-2/F2,6BPase, phosphofructokinase-2/fructose-2,6-bisphosphatase F2,6BP, fructose-2,6-bisphosphate; LHS, hormone-sensitive lipase. Other abbreviations are the same as used in Figs. 9.11, 9.12, and 9.14

The conversion of the key enzyme of glycogen degradation, GP, to its active form occurs through the phosphorylation of Ser14, catalyzed by PK (see Sect. 9.2.2). This enzyme, in turn, is also activated by phosphorylation, in this case catalyzed by PKA. Thus, PKA activation in response to glucagon binding to liver cells results in the phosphorylation of GP, which becomes active and promotes glycogen degradation (Fig. 9.18). The signaling pathway triggered by glucagon not only stimulates glycogen degradation but also impairs its synthesis. This is possible because the activity of the key enzyme in glycogen synthesis, glycogen synthase (GS), is also regulated by PKA-mediated phosphorylation (for details on glycogen synthesis pathway, see Sect. 8.2). However, the phosphorylation in this case causes the inhibition of the enzyme activity (Fig. 9.18). PKA can directly phosphorylate GS or can phosphorylate another protein kinase, the glycogen synthase kinase 3 (GSK3), which also phosphorylates GS. Thus, the hyperphosphorylation of GS impairs glycogen synthesis.

As discussed in Sect. 9.3.3, fructose-2,6-bisphosphate is a key molecule in the reciprocal regulation of glycolysis/gluconeogenesis. This compound is formed/degraded by the action of the bifunctional enzyme PFK-2/F2,6BPase, which is also a substrate for PKA. PKA-mediated phosphorylation of Ser32 of the bifunctional enzyme stimulates the F2,6BPase and inhibits the PFK-2 activities, leading to

fructose-2,6-bisphosphate degradation and, therefore, to activation of gluconeogenesis and inhibition of glycolysis (Fig. 9.18).

The uptake and the ability of the liver to use amino acids are also increased in response to glucagon. This seems to occur mainly at the transcriptional level, due to the induction of the expression of the genes encoding the hepatic alanine transporter and different transaminases, such as alanine aminotransferase, aspartate aminotransferase, and tyrosine aminotransferase. This control enables the more efficient use of the amino acids as precursors of glucose synthesis through the gluconeogenesis pathway.

Lipid metabolism in the liver is also regulated by PKA-dependent phosphorylation. The enzyme that has its activity regulated is acetyl-CoA carboxylase (ACC), a key enzyme in the fatty acid synthesis pathway (see Sect. 8.3). The phosphorylated ACC is inactive, impairing fatty acid synthesis. Additionally, malonyl-CoA, the product of the reaction catalyzed by ACC and an important intermediate of this pathway, is a potent inhibitor of the transport of acyl-CoA molecules across the mitochondria membranes. Since malonyl-CoA is not formed due to glucagon-mediated inhibition of ACC, liver β-oxidation can proceed during the hypoglycemia situation.

9.4.1.2 Effects of Glucagon on the Adipose Tissue

An important effect of glucagon on the adipose tissue is the activation of lipolysis, which ensures the increase of fatty acid concentration in the blood, making these molecules available to different tissues as the main energetic supply. The phosphorylation of the adipocyte enzyme hormone-sensitive lipase by PKA activates it, increasing the hydrolysis of triacylglycerols to glycerol and fatty acids, which are then released in the bloodstream (Fig. 9.18; see also Sect. 7.4.1). Glycerol may be used as substrate for gluconeogenesis, whereas fatty acids undergo β-oxidation in different tissues.

9.4.2 Glucocorticoids: Mechanism of Action and Effects on Energy Metabolism

Glucocorticoids are steroid hormones synthesized from cholesterol in the cortex of adrenal glands (Fig. 9.19). In humans, the main glucocorticoids produced are cortisol (80–90 %) and corticosterone.

The synthesis of the glucocorticoids is stimulated by the adrenocorticotropic hormone (ACTH), a peptidic hormone produced in the anterior pituitary gland. The release of ACTH by the pituitary is, in turn, stimulated by another hormone, the corticotropin-releasing hormone (CRH). This hormone is produced by the hypothalamus and reaches the pituitary through a portal system (Fig. 9.19). Thus, the synthesis and secretion of glucocorticoids by the adrenal glands are under control of the hypothalamic–pituitary–adrenal axis, in a classical example of the integration of nervous and endocrine systems. Furthermore, there is an efficient feedback control

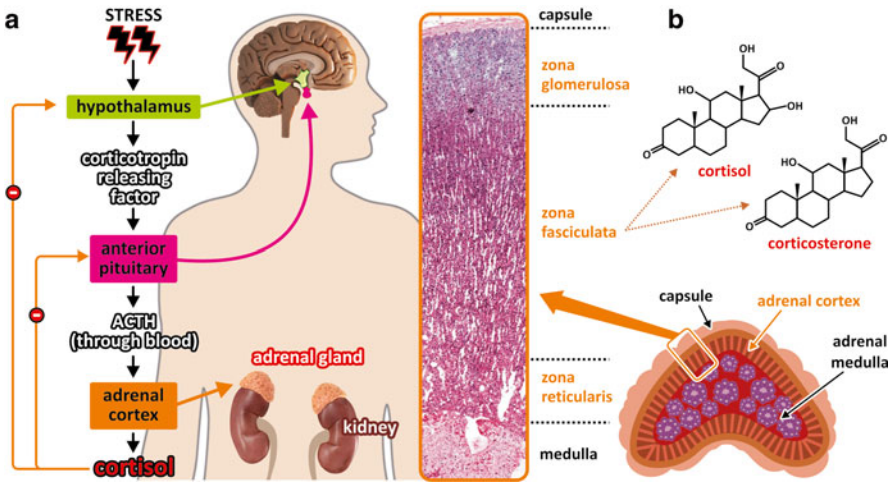


Fig. 9.19 (**a**) Hypothalamic–pituitary–adrenal axis and glucocorticoid secretion. (**b**) Representation of the adrenal gland, showing the two distinct parts: the adrenal cortex and the adrenal medulla and the three zones of the cortex, the zona glomerulosa, the zona fasciculata, and the zona reticularis. Cortisol and corticosterone, the natural human glucocorticoids, synthesized in the zona fasciculata and in the zona reticularis (The histological image was reprinted with the permission of Instituto de Histologia e Biologia do Desenvolvimento, Faculdade de Medicina, Universidade de Lisboa, FMUL)

of glucocorticoid production, in which the release of both ACTH and CRH is suppressed when circulating glucocorticoid levels reach a specific threshold.

Glucocorticoid secretion may be regulated in two levels. The first level is constitutive and follows the circadian rhythm, leading to a peak in cortisol concentration in the blood in the beginning of the morning. This pattern is adjusted by individual habits through the light/dark cycles. The second level corresponds to a response to virtually all types of physical or mental stress (Fig. 9.19), in which hypoglycemia can be included.

Due to their hydrophobic nature, the glucocorticoids enter the cells and bind to intracellular receptors (see Chap. 5). The hormone-receptor complex migrates to the nucleus, where it interacts with specific regions of the genome, inducing or repressing the expression of several genes.

Glucocorticoid receptor belongs to the superfamily of the nuclear hormones receptors, which also includes the receptors for mineralocorticoids, androgen, progesterone, thyroid hormones, retinoic acid, and retinol. The structure of all these receptors shows three distinct domains: an N-terminal domain that interacts with the DNA and/or with other transcriptional factors, a central domain with two zinc-finger motifs responsible for the recognition and binding to specific DNA sequences, and a C-terminal domain that binds to the hormone.

When not bound to the hormone, the glucocorticoid receptor associates to a multiprotein complex, which includes some of the heat-shock proteins (Hsp). The interaction with the glucocorticoid results in receptor dissociation from this

complex and in its hyperphosphorylation, which exposes nuclear localization sequences that allow the hormone-receptor complex, if in the cytosol, to migrate to the nucleus. In the nucleus, the hormone-receptor complex forms dimers and the DNA-binding segments become exposed, enabling the regulation of gene expression (Fig. 9.20). The sequences to which the glucocorticoid-receptor complex binds are small palindromic sequences of 15 nucleotides known as the glucocorticoid-responsive elements.

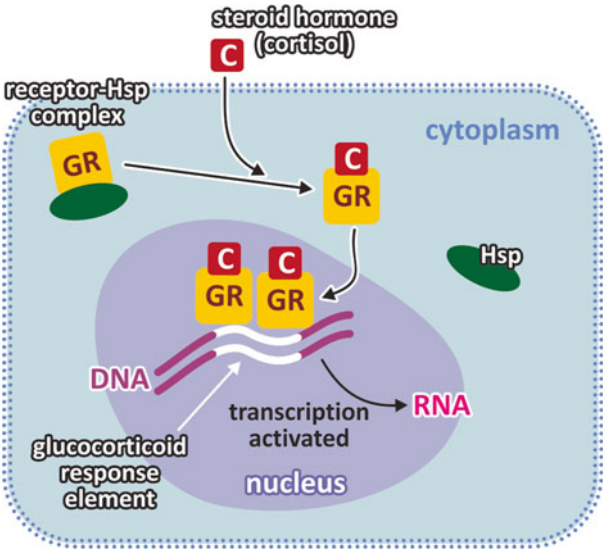


Fig. 9.20 Mechanism of action of the glucocorticoids. Intracellular binding of glucocorticoid to its receptor promotes receptor dissociation from its inhibitory multiprotein complex (Hsp - heat shock proteins) and ita migration to the nucleus where the hormone-receptor complex binds to segments in DNA containing the glucocorticoid response elements, leading to gene expression

9.4.2.1 Effects of Glucocorticoids on Muscle Metabolism

The regulation of protein and amino acid metabolisms by glucocorticoids are among the first actions of these hormones to be characterized. Glucocorticoids seem to have a crucial role in the mobilization of muscular proteins in hypoglycemia, yielding amino acids for liver and kidney gluconeogenesis. Indeed, several studies have shown that the glucocorticoids stimulate protein degradation. The mechanisms of this regulation are still not completely understood, but glucocorticoid-induced proteolysis seems to be restricted to the muscles.

Measurements of the arteriovenous differences in the muscles during fasting revealed that a net release of amino acids from the muscles occurred and, more interestingly, that 60 % of them were alanine and glutamine (Table 9.3), although

Table 9.3 Amino acid released from the skeletal muscle during fasting in man (based on data from Felig. Annu. Rev. Biochem. 44:933–955, 1975)

Amino acid	Arteriovenous difference (μmol/L)	Percentage of total
Alanine	−70	30
Glutamine	−70	30
Glycine	−24	10
Lysine	−20	9
Proline	−16	7
Threonine	−10	4
Histidine	−10	4
Leucine	−10	4
Valine	−8	3
Arginine	−5	2
Phenylalanine	−5	2
Tyrosine	−4	2
Methionine	−4	2
Isoleucine	−4	2
Cysteine	+10	–
Serine	+10	–

these two amino acids correspond to approximately 10 % of muscle proteins' content.

Therefore, it seems clear that the different amino acids produced by muscle proteolysis should be preferentially converted into Ala and Gln before being released from muscle cells. At this point, the role of glucocorticoids is very important. The genes encoding the different transaminases have glucocorticoid-responsive elements and, therefore, have their expression induced by these hormones. The increase in transaminase synthesis and consequently in their activities allows the interconversion of different amino acids into Ala and Glu (see Sect. 7.5), with the subsequent amination of Glu to generate Gln (Fig. 9.21).

Ala and Gln released from the muscles into the bloodstream during fasting or low-carbohydrate diets are used as precursors for gluconeogenesis. Quantitatively, Ala is the most important amino acid that is used as a gluconeogenic precursor in the liver, whereas Gln is preferentially used by the kidney (Fig. 9.21).

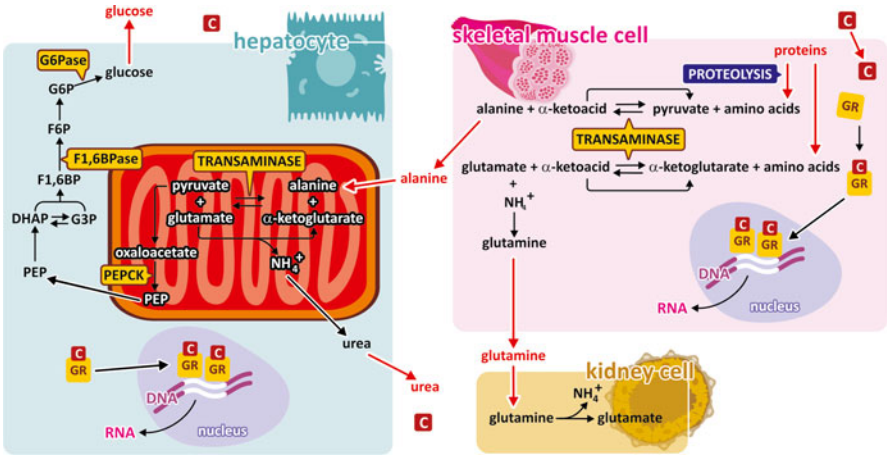


Fig. 9.21 Integration of the effects of glucocorticoids in different organs, showing the regulation of metabolic pathways in each cell type. The *red square* with a C represents cortisol; the *yellow squares* represent the glucocorticoid receptor; the enzymes in yellow boxes are the ones whose syntheses are induced by cortisol

9.4.2.2 Effects of Glucocorticoids on Liver Metabolism

The induction of the expression of the genes encoding the transaminases also occurs in the liver as a result of glucocorticoid action. This facilitates transamination of the available amino acids allowing them to enter gluconeogenesis (Fig. 9.21). Additionally, the genes of different gluconeogenic enzymes contain glucocorticoid-responsive elements and are under the control of these hormones, so that glucocorticoids induce the synthesis of PEPCK, F1,6BPase, and glucose-6-phosphatase, whose concentrations greatly increase in the liver cells. Thus, glucocorticoid action on liver cells enhances the hepatic capacity to perform gluconeogenesis.

However, it is important to take into account that glucocorticoid action alone is not sufficient to induce gluconeogenesis, since it is not only the presence of a given enzyme in high concentrations that will ensure its activation. Depending on the enzyme, changes in its phosphorylation state or the presence of an allosteric modulator is also necessary to allow the enzyme to operate. But since glucagon and glucocorticoids are both secreted as a response to hypoglycemia, in the case of gluconeogenesis, additive effects of these hormones occur, ensuring the activation of this metabolic pathway.

In addition, some effects of glucocorticoids represent another type of metabolic control. This is the case, for example, of their action on the genes encoding ACC

(a key enzyme in the synthesis of fatty acid) and GS (a key enzyme in the synthesis of glycogen). Glucocorticoids induce the expression of both enzymes, but the effect of glucagon, which is operating simultaneously, leads to the phosphorylation and inactivation of these enzymes. However, when glycemia increases (e.g., after a meal), glucagon action stops and these enzymes, in high concentration due to glucocorticoids effect, become active. Thus, the longer the period in hypoglycemia, the higher the concentrations of these enzymes. This will ensure the storage of the nutrient after a meal. Therefore, glucocorticoids prepare the organism to become more efficient in using the nutrients after a period of scarcity. Certainly, this role of glucocorticoids was very important for human beings in the past. However, today, when food is easily available, this adaptation to starvation may be one of the causes of the alarming increase in obesity in certain areas of the globe.

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