

chapter

15

Principles of Metabolic Regulation

1. Measurement of Intracellular Metabolite Concentrations Measuring the concentrations of metabolic intermediates in a living cell presents great experimental difficulties—usually a cell must be destroyed before metabolite concentrations can be measured. Yet enzymes catalyze metabolic interconversions very rapidly, so a common problem associated with these types of measurements is that the findings reflect not the physiological concentrations of metabolites but the equilibrium concentrations. A reliable experimental technique requires all enzyme-catalyzed reactions to be instantaneously stopped in the intact tissue so that the metabolic intermediates do not undergo change. This objective is accomplished by rapidly compressing the tissue between large aluminum plates cooled with liquid nitrogen ($-190\text{ }^{\circ}\text{C}$), a process called **freeze-clamping**. After freezing, which stops enzyme action instantly, the tissue is powdered and the enzymes are inactivated by precipitation with perchloric acid. The precipitate is removed by centrifugation, and the clear supernatant extract is analyzed for metabolites. To calculate intracellular concentrations, the intracellular volume is determined from the total water content of the tissue and a measurement of the extracellular volume.

The intracellular concentrations of the substrates and products of the phosphofructokinase-1 reaction in isolated rat heart tissue are given in the table below.

Metabolite	Concentration μM^*
Fructose 6-phosphate	87.0
Fructose 1,6-bisphosphate	22.0
ATP	11,400
ADP	1,320

Source: From Williamson, J.R. (1965) Glycolytic control mechanisms I: inhibition of glycolysis by acetate and pyruvate in the isolated, perfused rat heart. *J. Biol. Chem.* **240**, 2308–2321.

*Calculated as $\mu\text{mol/mL}$ of intracellular water.

- Calculate Q , $[\text{fructose 1,6-bisphosphate}][\text{ADP}]/[\text{fructose 6-phosphate}][\text{ATP}]$, for the PFK-1 reaction under physiological conditions.
- Given a $\Delta G'^{\circ}$ for the PFK-1 reaction of -14.2 kJ/mol , calculate the equilibrium constant for this reaction.
- Compare the values of Q and K'_{eq} . Is the physiological reaction near or far from equilibrium? Explain. What does this experiment suggest about the role of PFK-1 as a regulatory enzyme?

Answer

(a) The mass-action ratio, $Q = \frac{(22.0\ \mu\text{M})(1,320\ \mu\text{M})}{(87.0\ \mu\text{M})(11,400\ \mu\text{M})} = 0.0293$

S-174 Chapter 15 Principles of Metabolic Regulation

$$(b) \Delta G'^{\circ} = -RT \ln K'_{eq} \quad (RT \text{ at } 25^{\circ}\text{C} = 2.48 \text{ kJ/mol})$$

$$\ln K'_{eq} = -\Delta G'^{\circ}/RT$$

$$= -(-14.2 \text{ kJ/mol})/(2.48 \text{ kJ/mol})$$

$$= 5.73$$

$$K'_{eq} = e^{-5.73} = 308$$

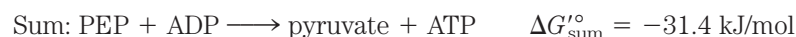
- (c) Because K'_{eq} is much greater than Q , it is clear that the PFK-1 reaction does not approach equilibrium in vivo, and thus the product of the reaction, fructose 1,6-bisphosphate, does not approximate an equilibrium concentration. Metabolic pathways are “open systems,” operating under near-steady state conditions, with substrates flowing in and products flowing out at all times. Thus, all the fructose 1,6-bisphosphate formed is rapidly used or turned over. The PFK-1-catalyzed reaction, which is the rate-limiting step in glycolysis, is an excellent candidate for the critical regulatory point of the pathway.

2. Are All Metabolic Reactions at Equilibrium?

- (a) Phosphoenolpyruvate (PEP) is one of the two phosphoryl group donors in the synthesis of ATP during glycolysis. In human erythrocytes, the steady-state concentration of ATP is 2.24 mM, that of ADP is 0.25 mM, and that of pyruvate is 0.051 mM. Calculate the concentration of PEP at 25 °C, assuming that the pyruvate kinase reaction (see Fig. 13–13) is at equilibrium in the cell.
- (b) The physiological concentration of PEP in human erythrocytes is 0.023 mM. Compare this with the value obtained in (a). Explain the significance of this difference.

Answer

- (a) First, we must calculate the overall $\Delta G'^{\circ}$ for the pyruvate kinase reaction by breaking this process into two reactions and summing the $\Delta G'^{\circ}$ values (from Table 13–6):



Assuming the reaction is at equilibrium, we can calculate K'_{eq} :

$$\Delta G'^{\circ} = -RT \ln K'_{eq}$$

$$\ln K'_{eq} = -\Delta G'^{\circ}/RT$$

$$= -(-31.4 \text{ kJ/mol})/(2.48 \text{ kJ/mol})$$

$$= 12.7$$

$$K'_{eq} = 3.28 \times 10^5$$

Because $K'_{eq} = \frac{[\text{pyruvate}][\text{ATP}]}{[\text{ADP}][\text{PEP}]}$, where all reactants and products are at their equilibrium concentrations,

$$\begin{aligned} [\text{PEP}] &= \frac{[\text{pyruvate}][\text{ATP}]}{[\text{ADP}] K'_{eq}} \\ &= \frac{(5.1 \times 10^{-5} \text{ M})(2.24 \times 10^{-3} \text{ M})}{(2.5 \times 10^{-4} \text{ M})(3.28 \times 10^5)} \\ &= 1.4 \times 10^{-9} \text{ M} \end{aligned}$$

- (b) The physiological [PEP] of 0.023 mM is

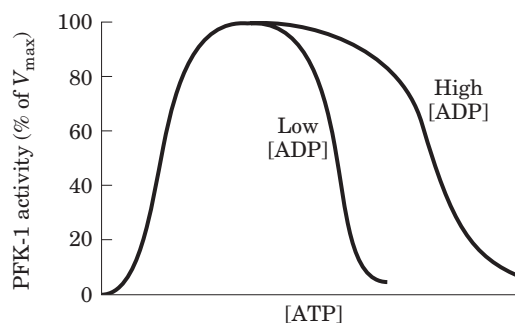
$$\frac{0.023 \times 10^{-3} \text{ M}}{1.4 \times 10^{-9} \text{ M}} = 16,000 \text{ times the equilibrium concentration}$$

This reaction, like many others in the cell, is *not* at equilibrium.

- 3. Effect of O₂ Supply on Glycolytic Rates** The regulated steps of glycolysis in intact cells can be identified by studying the catabolism of glucose in whole tissues or organs. For example, the glucose consumption by heart muscle can be measured by artificially circulating blood through an isolated intact heart and measuring the concentration of glucose before and after the blood passes through the heart. If the circulating blood is deoxygenated, heart muscle consumes glucose at a steady rate. When oxygen is added to the blood, the rate of glucose consumption drops dramatically, then is maintained at the new, lower rate. Explain.

Answer In the absence of O₂, the ATP needs of the cell are met by anaerobic glucose metabolism (fermentation) to form lactate. This produces a maximum of 2 ATP per glucose. Because the aerobic metabolism of glucose produces far more ATP per glucose (by oxidative phosphorylation), far less glucose is needed to produce the same amount of ATP. The Pasteur effect was the first demonstration of the primacy of energy production—that is, of ATP levels—in controlling the rate of glycolysis.

- 4. Regulation of PFK-1** The effect of ATP on the allosteric enzyme PFK-1 is shown below. For a given concentration of fructose 6-phosphate, the PFK-1 activity increases with increasing concentrations of ATP, but a point is reached beyond which increasing the concentration of ATP inhibits the enzyme.



- (a) Explain how ATP can be both a substrate and an inhibitor of PFK-1. How is the enzyme regulated by ATP?
- (b) In what ways is glycolysis regulated by ATP levels?
- (c) The inhibition of PFK-1 by ATP is diminished when the ADP concentration is high, as shown in the illustration. How can this observation be explained?

Answer

- (a) In addition to their binding sites for substrate(s), allosteric enzymes have binding sites for regulatory metabolites. Binding of effectors to these regulatory sites modifies the enzyme's activity by altering its V_{\max} or K_m value. ATP is a substrate and an allosteric inhibitor of PFK-1. Binding of ATP to the catalytic site increases activity, whereas binding to the allosteric site inhibits activity.
- (b) Because ATP is a negative regulator of PFK-1, elevation of [ATP] when energy is abundant inhibits the enzyme and thus the flux of metabolites through the glycolytic pathway.
- (c) The graph indicates that increased [ADP] suppresses the inhibition of PFK-1 by ATP. Because the total adenine nucleotide pool is more or less constant in all cells, utilization of ATP leads to an increase in [ADP]. The data show that the activity of the enzyme may be regulated in vivo by the ratio [ATP]/[ADP].

S-176 Chapter 15 Principles of Metabolic Regulation

- 5. Cellular Glucose Concentration** The concentration of glucose in human blood plasma is maintained at about 5 mM. The concentration of free glucose inside a myocyte is much lower. Why is the concentration so low in the cell? What happens to glucose after entry into the cell? Glucose is administered intravenously as a food source in certain clinical situations. Given that the transformation of glucose to glucose 6-phosphate consumes ATP, why not administer intravenous glucose 6-phosphate instead?

Answer Glucose enters cells and is immediately exposed to hexokinase, which converts glucose to glucose 6-phosphate using the energy of ATP. This reaction is highly exergonic ($\Delta G'^{\circ} = -16.7$ kJ/mol), and formation of glucose 6-phosphate is strongly favored. Glucose 6-phosphate is negatively charged and cannot diffuse across the membrane. The glucose transporter is specific for glucose; glucose 6-phosphate cannot leave the cell on this transporter and must be stored (by conversion to glycogen) or metabolized via glycolysis. Intravenous administration of glucose 6-phosphate is not useful because the phosphorylated glucose cannot enter cells on the glucose transporter or by diffusion.

- 6. Enzyme Activity and Physiological Function** The V_{\max} of the enzyme glycogen phosphorylase from skeletal muscle is much greater than the V_{\max} of the same enzyme from liver tissue.
- (a) What is the physiological function of glycogen phosphorylase in skeletal muscle? In liver tissue?
- (b) Why does the V_{\max} of the muscle enzyme need to be larger than that of the liver enzyme?

Answer

- (a) The role of glycogen and glycogen metabolism differs in muscle and liver. *In muscle*, glycogen is broken down to supply energy (ATP), via glycolysis and lactic acid fermentation. Glycogen phosphorylase catalyzes the conversion of stored glycogen to glucose 1-phosphate, which is converted to glucose 6-phosphate and thus enters glycolysis. During strenuous activity, muscle becomes anaerobic and large quantities of glucose 6-phosphate undergo lactic acid fermentation to form the necessary ATP.

In the liver, glycogen is used to maintain the level of glucose in the blood (primarily between meals). In this case, the glucose 6-phosphate is converted to glucose and exported into the bloodstream.

- (b) Strenuous muscular activity requires large amounts of ATP, which must be formed rapidly and efficiently, so glycogen phosphorylase must have a high ratio of V_{\max}/K_m in muscle. This is not a critical requirement in liver tissue.

- 7. Glycogen Phosphorylase Equilibrium** Glycogen phosphorylase catalyzes the removal of glucose from glycogen. The $\Delta G'^{\circ}$ for this reaction is 3.1 kJ/mol. (a) Calculate the ratio of $[P_i]$ to [glucose 1-phosphate] when the reaction is at equilibrium. (Hint: The removal of glucose units from glycogen does not change the glycogen concentration.) (b) The measured ratio $[P_i]/[\text{glucose 1-phosphate}]$ in myocytes under physiological conditions is more than 100:1. What does this indicate about the direction of metabolite flow through the glycogen phosphorylase reaction in muscle? (c) Why are the equilibrium and physiological ratios different? What is the possible significance of this difference?

Answer

- (a) First, we need to calculate the equilibrium constant (assuming a temperature of 25 °C):

$$\begin{aligned}\Delta G'^{\circ} &= -RT \ln K'_{\text{eq}} \\ \ln K'_{\text{eq}} &= -\Delta G'^{\circ}/RT \\ &= -(3.1 \text{ kJ/mol})/(2.48 \text{ kJ/mol}) \\ &= -1.2 \\ K'_{\text{eq}} &= e^{-1.2} = 0.30\end{aligned}$$

For the glycogen phosphorylase reaction:



$$K'_{eq} = \frac{[\text{glycogen}_{n-1}][\text{glucose 1-phosphate}]}{[\text{glycogen}_n][\text{P}_i]}$$

where all reactants are at their equilibrium concentrations. Because the concentration of glycogen remains constant, these terms cancel and the expression becomes

$$K'_{eq} = \frac{[\text{glucose 1-phosphate}]}{[\text{P}_i]}$$

This may be arranged to

$$\frac{[\text{P}_i]}{[\text{glucose 1-phosphate}]} = \frac{1}{K'_{eq}} = \frac{1}{0.3} = 3.3$$

(b) and **(c)** The high $[\text{P}_i]/[\text{glucose 1-phosphate}]$ ratio in myocytes ($>100:1$) means that $[\text{glucose 1-phosphate}]$ is far below the equilibrium value. The rate at which phosphoglucose mutase removes glucose 1-phosphate (by conversion to glucose 6-phosphate, which enters glycolysis) is greater than the rate at which glycogen phosphorylase can produce it. This indicates that the direction of metabolite flow is from glycogen to glucose-1-phosphate, and that the glycogen phosphorylase reaction is the rate-limiting step in glycogen breakdown.

- 8. Regulation of Glycogen Phosphorylase** In muscle tissue, the rate of conversion of glycogen to glucose 6-phosphate is determined by the ratio of phosphorylase *a* (active) to phosphorylase *b* (less active). Determine what happens to the rate of glycogen breakdown if a muscle preparation containing glycogen phosphorylase is treated with **(a)** phosphorylase kinase and ATP; **(b)** PP1; **(c)** epinephrine.

Answer

- (a)** Treatment with the kinase and ATP converts glycogen phosphorylase to the more active, phosphorylated form, phosphorylase *a*; glycogen breakdown accelerates.
- (b)** Treatment with the phosphatase PP1 converts the active phosphorylase *a* to the less active phosphorylase *b*; glycogen breakdown slows.
- (c)** Addition of epinephrine to muscle tissue causes the synthesis of cyclic AMP, which activates phosphorylase kinase. The kinase converts phosphorylase *b* (less active) to phosphorylase *a* (more active); glycogen breakdown accelerates.

- 9. Glycogen Breakdown in Rabbit Muscle** The intracellular use of glucose and glycogen is tightly regulated at four points. To compare the regulation of glycolysis when oxygen is plentiful and when it is depleted, consider the utilization of glucose and glycogen by rabbit leg muscle in two physiological settings: a resting rabbit, with low ATP demands, and a rabbit that sights its mortal enemy, the coyote, and dashes into its burrow. For each setting, determine the relative levels (high, intermediate, or low) of AMP, ATP, citrate, and acetyl-CoA and describe how these levels affect the flow of metabolites through glycolysis by regulating specific enzymes. In periods of stress, rabbit leg muscle produces much of its ATP by anaerobic glycolysis (lactate fermentation) and very little by oxidation of acetyl-CoA derived from fat breakdown.

Answer A primary role of glycolysis is the production of ATP, and the pathway is regulated to ensure efficient ATP formation. The utilization of glycogen and glucose to supply energy is regulated at the following steps: glycogen phosphorylase, phosphofructokinase-1, pyruvate kinase, and entry of acetyl-CoA into the citric acid cycle. In muscle, the primary regulatory metabolites are ATP, AMP, citrate, and acetyl-CoA. ATP is an inhibitor of glycogen phosphorylase and PFK-1; AMP stimulates both. Citrate inhibits PFK-1, and acetyl-CoA inhibits pyruvate kinase. Lack of O_2 leads to elevated levels of NADH, inhibiting pyruvate dehydrogenase and promoting fermentation of pyruvate to lactate.

S-178 Chapter 15 Principles of Metabolic Regulation

Under resting conditions, [ATP] is high and [AMP] low because the total adenine nucleotide pool is constant. [Citrate] and [acetyl-CoA] are intermediate because O_2 is not limiting and the citric acid cycle is functioning. Under conditions of active exertion (running), O_2 becomes limiting and ATP synthesis decreases. Consequently, [ATP] is relatively low and [AMP] relatively high, compared with aerobic conditions. [Citrate] and [acetyl-CoA] are low. These changes release the inhibition of glycolysis and stimulate lactic acid production.

- 10. Glycogen Breakdown in Migrating Birds** Unlike the rabbit with its short dash, migratory birds require energy for extended periods of time. For example, ducks generally fly several thousand miles during their annual migration. The flight muscles of migratory birds have a high oxidative capacity and obtain the necessary ATP through the oxidation of acetyl-CoA (obtained from fats) via the citric acid cycle. Compare the regulation of muscle glycolysis during short-term intense activity, as in the fleeing rabbit, and during extended activity, as in the migrating duck. Why must the regulation in these two settings be different?

Answer Migratory birds have a very efficient respiratory system to ensure that O_2 is available to flight muscles under stress (see Box 14–2). Birds also rely on the aerobic oxidation of fat, which produces the greatest amount of energy per gram of fuel. The migratory bird must regulate glycolysis so that glycogen is used only for short bursts of energy, not for the long-term stress of prolonged flight. The sprinting rabbit relies on breakdown of stored (liver) glycogen and anaerobic glycolysis for short-term production of ATP for muscle activity.

The regulation of these two means of ATP production is very different. Under aerobic conditions (see answer to Problem 9), glycolysis is inhibited by the relatively high [ATP], as acetyl-CoA units derived from fat feed into the citric acid cycle and ATP is produced by oxidative phosphorylation. Under anaerobic conditions, glycolysis is stimulated and metabolism of fats does not occur at an appreciable rate because [citrate] and [acetyl-CoA] are low and O_2 (the final acceptor of electrons in oxidative phosphorylation) is absent.

- 11. Enzyme Defects in Carbohydrate Metabolism** Summaries of four clinical case studies follow. For each case, determine which enzyme is defective and designate the appropriate treatment, from the lists provided at the end of the problem. Justify your choices. Answer the questions contained in each case study. (You may need to refer to information in Chapter 14.)

Case A The patient develops vomiting and diarrhea shortly after milk ingestion. A lactose tolerance test is administered. (The patient ingests a standard amount of lactose, and the glucose and galactose concentrations of blood plasma are measured at intervals. In individuals with normal carbohydrate metabolism, the levels increase to a maximum in about 1 hour, then decline.) The patient's blood glucose and galactose concentrations do not increase during the test. Why do blood glucose and galactose increase and then decrease during the test in healthy individuals? Why do they fail to rise in the patient?

Case B The patient develops vomiting and diarrhea after ingestion of milk. His blood is found to have a low concentration of glucose but a much higher than normal concentration of reducing sugars. The urine tests positive for galactose. Why is the concentration of reducing sugar in the blood high? Why does galactose appear in the urine?

Case C The patient complains of painful muscle cramps when performing strenuous physical exercise but has no other symptoms. A muscle biopsy indicates a muscle glycogen concentration much higher than normal. Why does glycogen accumulate?

Case D The patient is lethargic, her liver is enlarged, and a biopsy of the liver shows large amounts of excess glycogen. She also has a lower than normal blood glucose level. What is the reason for the low blood glucose in this patient?

Defective Enzyme

- (a) Muscle PFK-1
- (b) Phosphomannose isomerase
- (c) Galactose 1-phosphate uridylyltransferase
- (d) Liver glycogen phosphorylase

- (e) Triose kinase
- (f) Lactase in intestinal mucosa
- (g) Maltase in intestinal mucosa
- (h) Muscle debranching enzyme

Treatment

1. Jogging 5 km each day
2. Fat-free diet
3. Low-lactose diet
4. Avoiding strenuous exercise
5. Large doses of niacin (the precursor of NAD⁺)
6. Frequent feedings (smaller portions) of a normal diet

Answer

Case A: (f) Lactase in the intestinal mucosa hydrolyzes milk lactose to glucose and galactose, so the levels of these sugars increase transiently after milk ingestion. This increase would not occur in a person lacking lactase; the patient would show symptoms of lactose toxicity. The patient should exclude lactose (milk) from the diet (treatment **3**).

Case B: (c) Galactose 1-phosphate uridylyltransferase is an enzyme involved in conversion of galactose to glucose, which then can enter glycolysis. Absence of this enzyme leads to accumulation of galactose in the blood and excretion in the urine. A patient with this deficiency should be on a low-lactose diet (treatment **3**).

Case C: (h) Without the muscle form of glycogen phosphorylase, glycogen cannot be mobilized to supply ATP during extended exercise, and the result is weakness and cramping during the exercise. Because glycogen continues to be synthesized but is not mobilized, the muscle glycogen level is higher than normal. The patient should avoid strenuous exercise (treatment **4**).

Case D: (d) Liver glycogen functions as a source of blood glucose. Accumulation of glycogen and low blood glucose suggest that the liver glycogen phosphorylase is defective or of low activity. The patient should eat light meals regularly and frequently (treatment **6**).

12. Effects of Insufficient Insulin in a Person with Diabetes A man with insulin-dependent diabetes is brought to the emergency room in a near-comatose state. While vacationing in an isolated place, he lost his insulin medication and has not taken any insulin for two days.

- (a) For each tissue listed below, is each pathway faster, slower, or unchanged in this patient, compared with the normal level when he is getting appropriate amounts of insulin?
- (b) For each pathway, describe at least one control mechanism responsible for the change you predict.

Tissue and Pathways

1. Adipose: fatty acid synthesis
2. Muscle: glycolysis; fatty acid synthesis; glycogen synthesis
3. Liver: glycolysis; gluconeogenesis; glycogen synthesis; fatty acid synthesis; pentose phosphate pathway

Answer

- (a) (1) Adipose: fatty acid synthesis slower. (2) Muscle: glycolysis, fatty acid synthesis, and glycogen synthesis slower. (3) Liver: glycolysis faster; gluconeogenesis, glycogen synthesis, and fatty acid synthesis slower; pentose phosphate pathway unchanged.
- (b) (1) Adipose and (3) liver: fatty acid synthesis slower because lack of insulin results in inactive acetyl-CoA carboxylase, the first enzyme of fatty acid synthesis. Glycogen synthesis inhibited by cAMP-dependent phosphorylation (thus activation) of glycogen synthase. (2) Muscle: glycolysis slower because GLUT4 is inactive, so glucose uptake is inhibited. (3) Liver: glycolysis slower because the bifunctional PFK-2/FBPase-2 is

S-180 Chapter 15 Principles of Metabolic Regulation

converted to the form with active FBPase-2, decreasing [fructose 2,6-bisphosphate], which allosterically stimulates phosphofructokinase and inhibits FBPase-1; this also accounts for the stimulation of gluconeogenesis.

- 13. Blood Metabolites in Insulin Insufficiency** For the patient described in Problem 12, predict the levels of the following metabolites in his blood *before* treatment in the emergency room, relative to levels maintained during adequate insulin treatment: **(a)** glucose; **(b)** ketone bodies; **(c)** free fatty acids.

Answer

- (a)** Elevated; without insulin, muscle and other tissues cannot take up glucose, because the GLUT4 transporters do not move to the plasma membrane and ingested glucose remains in the blood.
- (b)** Elevated; accumulation of acetyl-CoA from fatty acid oxidation shifts the thiolase equilibrium toward acetoacetyl-CoA formation, the first step in ketone body formation.
- (c)** Elevated; to provide fuel for muscle and other tissues, fatty acids are mobilized from adipose and move through the blood to muscle and other tissues.

- 14. Metabolic Effects of Mutant Enzymes** Predict and explain the effect on glycogen metabolism of each of the following defects caused by mutation: **(a)** loss of the cAMP-binding site on the regulatory subunit of protein kinase A (PKA); **(b)** loss of the protein phosphatase inhibitor (inhibitor 1 in Fig. 15-40); **(c)** overexpression of phosphorylase *b* kinase in liver; **(d)** defective glucagon receptors in liver.

Answer

- (a)** PKA cannot be activated in response to glucagon or epinephrine, and glycogen phosphorylase is not activated.
- (b)** PP1 remains active, allowing it to dephosphorylate glycogen synthase (activating it) and glycogen phosphorylase (inhibiting it). Glycogen synthesis is stimulated, and glycogen breakdown inhibited.
- (c)** Phosphorylase remains phosphorylated (active), increasing the breakdown of glycogen and thus depleting the store of liver glycogen.
- (d)** Gluconeogenesis cannot be stimulated when blood glucose is low, leading to dangerously low blood glucose during periods of fasting.

- 15. Hormonal Control of Metabolic Fuel** Between your evening meal and breakfast, your blood glucose drops and your liver becomes a net producer rather than consumer of glucose. Describe the hormonal basis for this switch, and explain how the hormonal change triggers glucose production by the liver.

Answer The drop in blood glucose triggers release of glucagon by the pancreas. In the liver, glucagon activates glycogen phosphorylase by stimulating its cAMP-dependent phosphorylation and stimulates gluconeogenesis by lowering [fructose 2,6-bisphosphate], thus stimulating FBPase-1, a key enzyme in gluconeogenesis.

- 16. Altered Metabolism in Genetically Manipulated Mice** Researchers can manipulate the genes of a mouse so that a single gene in a single tissue either produces an inactive protein (a “knockout” mouse) or produces a protein that is always (constitutively) active. What effects on metabolism would you predict for mice with the following genetic changes: **(a)** knockout of glycogen debranching enzyme in the liver; **(b)** knockout of hexokinase IV in liver; **(c)** knockout of FBPase-2 in liver; **(d)** constitutively active FBPase-2 in liver; **(e)** constitutively active AMPK in muscle; **(f)** constitutively active ChREBP in liver?

Answer

- (a) Reduced capacity to mobilize glycogen; lowered blood glucose between meals
- (b) Reduced capacity to lower blood glucose after a carbohydrate meal; elevated blood glucose (see Fig. 15-12)
- (c) Reduced fructose 2,6-bisphosphate (F26BP) in liver, stimulating glycolysis and inhibiting gluconeogenesis (see Fig. 15-15)
- (d) Reduced F26BP, stimulating gluconeogenesis and inhibiting glycolysis
- (e) Increased uptake of fatty acids and glucose; increased oxidation of both (see Fig. 15-6)
- (f) Increased conversion of pyruvate to acetyl-CoA; increased fatty acid synthesis

Data Analysis Problem

17. Optimal Glycogen Structure Muscle cells need rapid access to large amounts of glucose during heavy exercise. This glucose is stored in liver and skeletal muscle in polymeric form as particles of glycogen. The typical glycogen particle contains about 55,000 glucose residues (see Fig. 15-33b). Meléndez-Hevia, Waddell, and Shelton (1993) explored some theoretical aspects of the structure of glycogen, as described in this problem.

- (a) The cellular concentration of glycogen in liver is about $0.01 \mu\text{M}$. What cellular concentration of free glucose would be required to store an equivalent amount of glucose? Why would this concentration of free glucose present a problem for the cell?

Glucose is released from glycogen by glycogen phosphorylase, an enzyme that can remove glucose molecules, one at a time, from one end of a glycogen chain. Glycogen chains are branched (see Figs 15-26 and 15-33b), and the degree of branching—the number of branches per chain—has a powerful influence on the rate at which glycogen phosphorylase can release glucose.

- (b) Why would a degree of branching that was too low (i.e., below an optimum level) reduce the rate of glucose release? (Hint: Consider the extreme case of no branches in a chain of 55,000 glucose residues.)
- (c) Why would a degree of branching that was too high also reduce the rate of glucose release? (Hint: Think of the physical constraints.)

Meléndez-Hevia and colleagues did a series of calculations and found that two branches per chain (see Fig. 15-33b) was optimal for the constraints described above. This is what is found in glycogen stored in muscle and liver.

To determine the optimum number of glucose residues per chain, Meléndez-Hevia and coauthors considered two key parameters that define the structure of a glycogen particle: t = the number of tiers of glucose chains in a particle (the molecule in Fig. 15-33b has five tiers); g_c = the number of glucose residues in each chain. They set out to find the values of t and g_c that would maximize three quantities: (1) the amount of glucose stored in the particle (G_T) per unit volume; (2) the number of unbranched glucose chains (C_A) per unit volume (i.e., number of chains in the outermost tier, readily accessible to glycogen phosphorylase); and (3) the amount of glucose available to phosphorylase in these unbranched chains (G_{PT}).

- (d) Show that $C_A = 2^{t-1}$. This is the number of chains available to glycogen phosphorylase before the action of the debranching enzyme.
- (e) Show that C_T , the total number of chains in the particle, is given by $C_T = 2^t - 1$. Thus $G_T = g_c(C_T) = g_c(2^t - 1)$, the total number of glucose residues in the particle.
- (f) Glycogen phosphorylase cannot remove glucose from glycogen chains that are shorter than five glucose residues. Show that $G_{PT} = (g_c - 4)(2^{t-1})$. This is the amount of glucose readily available to glycogen phosphorylase.
- (g) Based on the size of a glucose residue and the location of branches, the thickness of one tier of glycogen is $0.12g_c \text{ nm} + 0.35 \text{ nm}$. Show that the volume of a particle, V_s , is given by the equation $V_s = \frac{4}{3}\pi t^3(0.12g_c + 0.35)^3 \text{ nm}^3$.

