Hormonal Control of Glucose Metabolism

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Plasma glucose concentration is normally maintained within a narrow range despite wide fluctuations in the supply (meal) and demand (exercise) for nutrients. In adult humans, plasma glucose concentrations throughout a 24-hour period average 90 mg/dl, with maximum values 60–90 minutes after meals, usually not exceeding 140 mg/dl, and values during a moderate fast or exercise usually remaining above 50 mg/dl. This relative stability contrasts with the situation for other fuels such as glycerol, lactate, free fatty acids (FFA), and ketone bodies (acetoacetate and β-hydroxybutyrate), the fluctuations of which vary more widely. The reason why plasma glucose concentration must be maintained in a narrow range of concentration is related to the deleterious effects of both hypoglycemia and hyperglycemia. Acute hypoglycemia is well known to have harmful effects on the brain. Glucose is usually the main fuel used by the brain; this is because plasma concentrations of one of the alternative fuels (ketone bodies) are low, while transport across the blood-brain barrier of the other alternative fuel (free fatty acids) is limited. As the brain has low energy stores, it is markedly dependent upon circulating glucose for its energy metabolism. When plasma glucose levels fall below 60 mg/dl, brain glucose uptake decreases and cerebral function is impaired. More severe and prolonged hypoglycemia can cause convulsions, permanent brain damage, and death. Chronic hyperglycemia also has deleterious effects. The functional and vascular changes in the eyes, nerves, aorta, and kidneys of diabetic patients seem to be related to increased metabolism of glucose via the polyol pathway. To maintain plasma glucose concentrations within a normal range, the changes in the dietary or endogenous glucose supply must be precisely matched by comparable changes in tissue uptake. Conversely, the changes in tissue glucose uptake (for example, during exercise) must be matched by appropriate changes in the exogenous or endogenous glucose supply.

The aim of this chapter is to review briefly the mechanisms by which the rate of glucose production and uptake is finely regulated during a 24-hour period in normal resting humans, and how they are coordinated by hormones and alternative substrates to maintain the plasma glucose level within a normal range. The cellular mechanisms by which hormones alter the rate of hepatic glucose production and peripheral tissue glucose utilization will also briefly be discussed.
OVERALL GLUCOSE HOMEOSTASIS IN HUMANS

In a normal human living in a Western society, the 24 hours of a normal day can be divided into three periods of approximately 4 hours, corresponding to the absorption and assimilation of the principal meals and to a postabsorptive period corresponding to the 12-hour overnight fast. During these periods, systemic glucose homeostasis is profoundly modified, as described in a recent review (1).

The Postabsorptive State

The postabsorptive period generally refers to the 12 hours following the last meal of the day during which transition from the fed to the fasting state occurs. At the end of this period, tissue glucose utilization in resting humans is approximately 2 mg/kg/min. Approximately 1 mg/kg/min is due to the obligatory uptake of glucose by the brain and other non-insulin-dependent tissues. Glucose uptake by insulin-dependent tissues (skeletal muscle, adipose tissue) accounts for less than 30–50% of glucose utilization. Tissue glucose uptake is precisely matched by the liver glucose output, and plasma glucose concentration remains constant.

The Postprandial State

Postprandial hyperglycemia is dependent upon the amount and form of carbohydrate ingested and on the amount of accompanying protein and fat [reviewed in (1)]. In general, complex carbohydrates are absorbed more slowly than simple sugars, and protein and fat delay absorption; both of these factors reduce postprandial glucose excursions. The time of the day when carbohydrates are ingested is also important since glucose tolerance is better in the morning than in the evening.

After a carbohydrate meal, plasma glucose levels increase after 15 minutes, glucose production is inhibited, and glucose utilization is enhanced. Plasma glucose, glucose production, and glucose utilization return to basal levels after 180 minutes. These changes are associated with a parallel increase in plasma insulin and a decrease in plasma glucagon. Plasma glucose concentrations after a meal are determined by the relative changes in the rates of glucose delivery and removal. The magnitude of these changes is largely determined by the secretion of insulin and glucagon.

The major tissues responsible for glucose removal after a carbohydrate meal are the liver, small intestine, brain, skeletal muscles, and adipose tissue [reviewed in (1)]. Skeletal muscles and splanchnic tissues (liver, small intestine) each probably account for 30%, brain for 20%, and adipose tissue for 10% of glucose taken up. The uptake of glucose by skeletal muscles and adipose tissue is influenced by both plasma glucose and insulin levels, whereas the uptake of glucose by the brain is only influenced by plasma glucose levels. Adipose tissue lipolysis and lipid oxidation are suppressed after carbohydrate ingestion. Of the glucose taken up by tissues, 60% is used for replenishment of liver and muscle glycogen stores, 30% is oxidized, and 10% is
released as lactate into the circulation for further uptake by the liver for indirect glycogen formation.

CONTROL OF HEPATIC GLUCOSE PRODUCTION

The only tissues that contain significant amounts of glucose-6-phosphatase, the enzyme necessary for hydrolysis of glucose-6-phosphate to glucose and the subsequent release of glucose into the circulation, are liver and kidney. The liver is the main source of circulating glucose except in two situations: 1. after a prolonged fast, when kidney may provide up to 10% of circulating glucose; 2. after meals or administration of exogenous nutrients (for example, intravenous infusions, parenteral nutrition).

The liver provides glucose to the circulation through two metabolic pathways: 1. glycogenolysis, the breakdown of glycogen stores, and 2. gluconeogenesis, the formation of new glucose molecules from amino acids, glycerol, and lactate. The contribution of each of these pathways in humans has been estimated from different types of studies: the rate of decrease in glycogen from serial liver biopsies, balance of gluconeogenic precursors across the splanchnic bed, and incorporation of isotopically labeled gluconeogenic precursors into circulating glucose [reviewed in (1)]. It was initially estimated that glycogenolysis accounts for 70% of overall hepatic glucose output in the postabsorptive period. However, recent studies employing nuclear magnetic resonance to measure depletion of hepatic glycogen stores suggest that glycogenolysis may account for only 30% of overall hepatic glucose output (2). If fasting is prolonged to 48–60 hours, gluconeogenesis accounts for virtually all of the hepatic glucose output.

On a minute-to-minute basis, glucose, insulin, and glucagon are the major factors regulating hepatic glucose production. After ingestion of a meal, plasma insulin and glucose concentrations increase, plasma glucagon levels are suppressed, and hepatic glucose production is reduced. Conversely, as one proceeds from the fed into the fasted state, plasma insulin and glucose levels decrease, plasma glucagon levels increase, and hepatic glucose production is increased. Under stressful conditions, circulating epinephrine from the adrenal medulla and neurally released norepinephrine become involved and augment hepatic glucose production through β-adrenergic receptors in humans (3). Glucocorticoids have no direct effects on hepatic glucose production, but they markedly potentiate the effects of glucagon and adrenaline (permissive role).

By using the "pancreatic clamp" technique, it has been possible to delineate the respective role of glucose and different hormones in hepatic glucose production in dogs in the postabsorptive state [reviewed in (4–6)]. This technique involves the infusion of somatostatin into a peripheral vein to inhibit the secretion of insulin and glucagon and the infusion of the two pancreatic hormones into the portal vein to replace their endogenous secretion. It is thus possible to fix the levels of plasma glucose, insulin, glucagon, catecholamines, and cortisol to the desired values. The
rates of liver glucose production and net hepatic glucose uptake are assessed by radioactive tracer and arteriovenous difference techniques. The principal conclusions from these experiments [reviewed in (4–6)] will be summarized briefly below and in Table 1.

**Role of Glucose in Hepatic Glucose Production**

It has been clearly shown that changes in plasma glucose concentrations can regulate hepatic glycogenolysis independently of pancreatic hormones. Moderate (200 mg/dl) or marked (300 mg/dl) hyperglycemia induced a complete inhibition of hepatic glucose production in postabsorptive dogs. Since gluconeogenesis represents only 10–20% of overall glucose production in postabsorptive dogs, hyperglycemia acts mainly by inhibiting glycogenolysis. Gluconeogenesis is much less sensitive than glycogenolysis to inhibition by hyperglycemia.

**Role of Glucagon in Hepatic Glucose Production**

In postabsorptive dogs, selective glucagon deficiency induced a 70% decrease in hepatic glucose production, suggesting that two-thirds of the production is attributable to glucagon. A selective physiological increase in portal plasma glucagon brought about a rapid and marked increase in hepatic glucose production that, however, waned with time. The evanescent effect, despite raised plasma glucagon levels, was due to glycogenolysis since gluconeogenesis remained increased. The evanescent effect is probably due to the hyperglycemia that developed and counteracted the effect of glucagon on glycogenolysis. Dose-response studies have indicated that the levels of glucagon required for half maximum activation of glycogenolysis and gluconeogenesis in vivo are similar and coincide with the commonly occurring physiologic increases of glucagon.

**Role of Catecholamines in Hepatic Glucose Production**

A selective physiological increase in plasma epinephrine or norepinephrine increased hepatic glucose production moderately and transiently. The evanescent effect, despite increased plasma epinephrine or norepinephrine levels, was due to the
ensuing hyperglycemia that counteracted the effect of epinephrine on glycogenolysis. The difference between epinephrine and norepinephrine was related to their effect on glycogenolysis. Norepinephrine did not increase glycogenolysis, whereas epinephrine stimulated it. In contrast, norepinephrine was more potent than epinephrine at stimulating gluconeogenesis.

Role of Insulin in Hepatic Glucose Production

A selective severe insulin deficiency brought about a rapid and marked increase in hepatic glucose production that, however, waned with time. Selective hypoinsulinemia also caused a progressive increase in gluconeogenesis. The evanescent effect, despite low plasma insulin levels, was due to the hyperglycemia that developed and counteracted the effect of insulin deficiency on glycogenolysis. A selective physiological increase in portal plasma insulin rapidly and efficiently inhibited hepatic glucose production. As hyperinsulinemia was ineffective in inhibiting gluconeogenesis, it has been suggested that gluconeogenesis is inhibited at low plasma insulin levels and that small decrement of plasma insulin markedly enhance this process. Dose-response studies have indicated that the levels of insulin required for half maximum inhibition of glycogenolysis in vivo are much lower than the levels of insulin required for half maximum inhibition of gluconeogenesis.

Mechanisms Involved in the Control of Hepatic Glucose Production

Control of Hepatic Glycogenolysis

The rate-limiting step in hepatic glycogenolysis is catalyzed by phosphorylase. This enzyme catalyzes the hydrolysis of 1,4 linkages of glycogen to yield glucose-1-phosphate. In liver, phosphorylase exists in both an active and an inactive form (Fig. 1). Active phosphorylase (phosphorylase a) is phosphorylated on a serine residue by phosphorylase kinase. Inactive phosphorylase (phosphorylase b) is dephosphorylated by a specific protein phosphatase-1. Phosphorylase kinase also exists in both an active and an inactive form. Active phosphorylase kinase (phosphorylase kinase a) is phosphorylated on a serine residue by the cyclic adenosine monophosphate (cAMP)-dependent protein kinase. Inactive phosphorylase kinase (phosphorylase kinase b) is dephosphorylated by a specific protein phosphatase-1.

The molecular mechanisms by which glucose is able to inhibit liver glycogenolysis involve the binding of glucose to phosphorylase a and its inactivation. In addition, when phosphorylase a has bound glucose, it is a much better substrate for protein phosphatase-1. The effect of high glucose concentration is, therefore, to cause the conversion of phosphorylase b to phosphorylase a and to arrest glycogenolysis. Furthermore, since phosphorylase a is a potent inhibitor of synthase phosphatase, its inhibition allows the latter enzyme to activate glycogen synthase and to initiate glycogen synthesis after a lag period of a few minutes.
The molecular mechanisms by which glucagon stimulates glycogenolysis are relatively well known. After binding to plasma membrane receptors, glucagon induces an increase in intracellular cAMP levels. cAMP then binds to the regulatory subunit of cAMP-dependent protein kinase and releases its catalytic subunit. The catalytic subunit of cAMP-dependent protein kinase stimulates the phosphorylation of phosphorylase kinase and activates phosphorylase kinase. Active phosphorylase kinase then stimulates the phosphorylation of phosphorylase and increases glycogenolysis.

In humans, exogenous catecholamines act mainly via β-adrenergic receptors and thus activate phosphorylase and stimulate glycogen breakdown by mechanisms similar to those described above for glucagon. In rodents, it has been shown that catecholamines act mainly via α-adrenergic receptors and stimulate glycogen breakdown via a cAMP-independent mechanism. Catecholamines, via α-adrenergic receptors, activate plasma membrane phospholipase C, which hydrolyses phosphatidylinositol 4,5-diphosphate into inositol 1,4,5-triphosphate and 1,2-diacylglycerol. Inositol 1,4,5 triphosphate is a potent stimulator of calcium mobilization from intracellular calciosomes. The increase in intracellular calcium stimulates phosphorylase kinase, a calmodulin-sensitive protein kinase.
The mechanism by which insulin inhibits phosphorylase and glycogen breakdown has not been entirely clarified. After binding to its receptor, insulin activates the tyrosine kinase activity of the $\beta$ subunit of the receptor, leading to the activation of a serine/threonine kinase by an unknown mechanism. An insulin-stimulated protein kinase has recently been identified in skeletal muscle (7). This enzyme is phosphorylated in response to insulin, leading to an activation of protein phosphatase-1 via phosphorylation on serine/threonine residues. Activated protein phosphatase-1 then stimulates the dephosphorylation of glycogen synthase and phosphorylase kinase, leading to an inhibition of glycogenolysis and an activation of glycogen synthesis. If this enzyme is present in liver and is activated by insulin, it could be involved in the inhibition of glycogenolysis.

Control of Hepatic Gluconeogenesis

Flux through the gluconeogenic pathway is regulated by different factors: 1. the provision of gluconeogenic substrates to the liver from peripheral tissues, 2. the activities of liver key enzymes that are influenced by substrates and hormones, and 3. by the provision of ATP and cofactors essential for these enzymes.

The major gluconeogenic precursors are lactate, amino acids, and glycerol. The supply of gluconeogenic precursor to the liver is regulated by various hormones (4,8). In the postabsorptive state and in most catabolic situations, amino acids (mainly glutamine and alanine) are released from skeletal muscle and glycerol is produced by adipose tissue. It has clearly been established that 1. insulin inhibits, whereas glucocorticoids stimulate, muscle proteolysis, and 2. insulin inhibits, whereas catecholamines (via $\beta$-adrenergic receptors) and growth hormone stimulate, adipose tissue lipolysis. In addition, glucocorticoids potentiate the lipolytic effect of catecholamines on adipose tissue. The regulation of lactate production by peripheral tissues is much less well known. The increased rate of free fatty acid delivery to peripheral tissues in the postabsorptive state and in most catabolic situations could play an important role, since fatty acids inhibit glucose oxidation and increase the release of lactate. Thus both hormones and free fatty acids play a crucial role in the regulation of delivery of gluconeogenic substrate to the liver. Nevertheless, several different hormones have been shown to have a marked effect on the regulation of gluconeogenesis when the gluconeogenic supply is maintained at a constant level, such as in the perfused liver or in isolated hepatocytes (4,8). This indicates that crucial steps of liver gluconeogenesis and/or glycolysis are regulated. Gluconeogenesis cannot be viewed as a reversal of glycolysis since three steps of glycolysis, catalyzed by glucokinase, phosphofructokinase, and pyruvate kinase, are characterized by a large and negative free-energy charge and are essentially irreversible in vivo. These three steps are bypassed by a separate set of enzymes, catalyzing different reactions that function in gluconeogenesis (Fig. 2). The regulatory enzymatic steps controlling net flux along the gluconeogenic pathway are pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose-1,6-diphosphatase, and glucose-6-phosphatase (9). Thus both glycolysis and gluconeogenesis are irreversible processes in
FIG. 2. Schematic representation of the pathway of glycolysis and gluconeogenesis in the liver. PEP, phosphoenolpyruvate; PEPCK, phosphoenolpyruvate carboxykinase.
FIG. 3. Schematic representation of the short-term control of liver gluconeogenesis. Fructose-1,6-P, fructose-1,6-phosphate; fructose-2,6-P2, fructose-2,6-bisphosphate; cAMP, cyclic adenosine monophosphate; 6-PF-2-K/F-2,6-Pase, 6-phosphofructo-2 kinase/fructose 2,6-bisphosphatase; PEP, phosphoenolpyruvate.
cells that are independently regulated through controls exerted on specific enzymatic steps that are not common to the two pathways.

It has been clearly established that gluconeogenesis is subject to short-term regulation by hormones at the level of the fructose-6-phosphate—fructose 1,6-bisphosphate and phosphoenolpyruvate—pyruvate cycles (10,11). Glucagon and β-adrenergic agents increase liver cAMP and activate cAMP-dependent protein kinase. The catalytic subunit of cAMP-dependent protein kinase stimulates the phosphorylation of L-type pyruvate kinase and inactivates this enzyme. The inhibition of pyruvate kinase decreases the conversion of phosphoenolpyruvate (PEP) to pyruvate and allows more PEP to be directed through the gluconeogenic pathway (Fig. 3). In addition, the catalytic subunit of cAMP-dependent protein kinase stimulates the phosphorylation of a bifunctional enzyme (6-phosphofructo-2-kinase/fructose 2,6-bisphosphatase) and inactivates it (12–14). This produces a decrease in the level of fructose 2,6-bisphosphate, an activator of phosphofructokinase and an inhibitor of fructose-1,6-bisphosphatase. The resulting reduction in intrahepatic fructose-2,6-bisphosphate level inhibits glycolysis and promotes gluconeogenesis (Fig. 3). Insulin antagonizes the action of low concentrations of glucagon on pyruvate kinase and 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase, via a decrease in liver cAMP and fructose-2,6-bisphosphate.

Another important factor in the short-term regulation of liver gluconeogenesis is the alteration in fatty acid oxidation (11). A rise in fatty acid oxidation increases the acetyl-CoA/CoA and NADH/NAD⁺ ratios and the ATP concentration. The increased mitochondrial acetyl-CoA leads to activation of pyruvate carboxylase, and the increased cytosolic NADH/NAD⁺ ratio allows displacement of the equilibrium reaction catalyzed by the glyceraldehyde phosphate dehydrogenase in the direction of gluconeogenesis (Fig. 4).

Various recent studies have also shown that glucagon and β-adrenergic agents (via cAMP), glucocorticoids, insulin, and glucose modulate the expression of several

![Diagram](https://example.com/diagram.png)

FIG. 4. Schematic representation of the role of free fatty acid oxidation in the regulation of liver gluconeogenesis. 1: pyruvate carboxylase; 2: phosphoenolpyruvate carboxykinase; 3: glyceraldehyde-3-phosphate dehydrogenase; 4: phosphoglycerate dehydrogenase. OAA, oxaloacetate; PEP, phosphoenolpyruvate; 3-PGA, 3-phosphoglycerate; 1,3-DPG, 1,3-diphosphoglycerate; F-1,6-P₂, fructose-1,6-bisphosphate; F-6-P, fructose-6-phosphate; G-6-P, glucose-6-phosphate; NADH, reduced nicotinamide adenine dinucleotide; GTP, guanosine triphosphate.
TABLE 2. Effect of hormones and substrates on expression of genes involved in hepatic carbohydrate metabolism

<table>
<thead>
<tr>
<th>Gene</th>
<th>Glucose</th>
<th>Insulin</th>
<th>Glucagon</th>
<th>Glucocorticoids</th>
<th>Thyroid hormones</th>
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<td>Glycolysis</td>
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<td>Glucokinase</td>
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<tr>
<td>6-PF-2K/F-2,6-Pase</td>
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<td>↑P</td>
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<td>Pyruvate kinase</td>
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<tr>
<td>Gluconeogenesis</td>
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<td>PEPCK</td>
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<td>Fructose-1,6-diphosphatase</td>
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<td>Glucose-6-phosphatase</td>
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6-PF-2K/F-2,6-Pase, 6-phosphofructo-2 kinase/Fructose 2,6 biphosphatase; PEPCK, phosphoenolpyruvate carboxykinase; P, permissive effect.

genes involved in the control of liver gluconeogenesis and glycolysis (15–17) (Table 2). It has been shown that cAMP increases the transcription of phosphoenolpyruvate carboxykinase (PEPCK) (15) and inhibits the transcription of glucokinase (18) and pyruvate kinase (17). Insulin inhibits the transcription of PEPCK (15) and increases the transcription of glucokinase (18). Glucose increases the transcription of pyruvate kinase, an effect that is potentiated by insulin (17).

Glucose-6-phosphatase is a microsomal enzyme that catalyzes the hydrolysis of glucose-6-phosphate to free glucose. The short-term regulation of this enzyme is not well known. It has been suggested that the enzyme is mainly controlled by the level of glucose-6-phosphate. The rise in glucose-6-phosphate in response to glucagon or catecholamines is sufficient to account for an increased flux through glucose-6-phosphatase with a concomitant increase in hepatic glucose production. In contrast, the fall in glucose-6-phosphate in response to an oral glucose load is sufficient to account for a diminished flux through glucose-6-phosphatase, with concomitant suppression of hepatic glucose production.

In summary, key factors affecting acute regulation of gluconeogenesis in humans include precursor supply, glucagon, insulin and lipid oxidation by the liver. Catecholamines released under stressful conditions can directly increase gluconeogenesis but probably exert their main action by providing additional precursors (for example glycerol and amino acids) and increasing intrahepatic lipid oxidation. Adrenocorticosteroids increase gluconeogenesis on a longer-term basis by increasing substrate supply and promoting increased synthesis of gluconeogenic enzyme. Their effects are synergistic with those of glucagon and catecholamines. The role of growth hormone remains to be defined.

CONTROL OF GLUCOSE UTILIZATION

The primary determinants of glucose utilization in vivo are plasma glucose concentration and, for certain tissues, plasma insulin concentration and the sensitivity of
tissues to insulin. The sensitivity of tissues to insulin can be affected by hormones such as cortisol, growth hormone and catecholamines (Table 1) and also by changes in the availability of alternative substrates (for example, free fatty acids and ketone bodies). After an oral glucose load, skeletal muscles and liver probably account for 60% of glucose taken up. Thus the metabolism of glucose in these tissues will be discussed.

Control of Muscle Glucose Metabolism

Uptake of glucose by most tissues occurs by facilitated diffusion, a process that is not energy dependent and that follows Michaelis-Menten kinetics. Transport kinetics vary among tissues, depending to a large extent on the characteristics of glucose transporters expressed in these tissues and on whether the process is sensitive to insulin. Five glucose transporter isoforms have been identified, the distribution of which varies among tissues, as does their dependency on insulin (19). One of them, GLUT-4, is expressed in insulin-dependent tissue (muscle and adipose tissue) and has a specific role in the regulation of glucose utilization in response to insulin. Insulin controls the transport of glucose in muscle and adipose cells by two different mechanisms. It acutely accelerates glucose transport by promoting the translocation of GLUT-4 glucose transporters from an intracellular store to the cell membrane (translocation mechanism) (20). It also controls the concentration of GLUT-4 glucose transporters by promoting their synthesis (21,22).

Glucose taken up by muscle cells has two main fates: storage as glycogen or conversion to pyruvate (Fig. 5). Pyruvate can then be converted to lactate, which is released into the circulation, or it can be oxidized to CO₂ (Fig. 5). The relative proportion of glucose that is metabolized in these different pathways varies between tissues and is dependent upon the hormonal environment and the presence of alternative substrates, for example, free fatty acids or ketone bodies. Thus, in the postabsorptive state there is no net storage of glucose, and all glucose taken up is either completely oxidized or converted to lactate (or alanine) which can be recycled back to the liver to be used for gluconeogenesis. The glucose-lactate and glucose-alanine cycles account for 25–35% of glucose uptake in the postabsorptive state (23,24). In the brain, almost all of the glucose taken up is completely oxidized, whereas in skeletal muscle most of the glucose taken up undergoes glycolysis and is recycled back to the liver as either lactate or alanine. In contrast, after an oral glucose load, a significant part of glucose is stored as glycogen.

Mechanisms Involved in the Control of Glycolysis

Glycolysis involves the anaerobic breakdown of glucose to pyruvate and lactate. When oxygen is not available, the reduced NADH, which is formed during glycolysis but cannot readily be oxidized by the respiratory chain, is oxidized by the lactate
dehydrogenase reaction, which catalyzes the conversion of pyruvate to lactate, thereby allowing glycolysis to proceed.

After the uptake of glucose into cells, there are numerous potential control points for glycolysis. The first is the phosphorylation of glucose to form glucose-6-phosphate. In non-hepatic tissues, this reaction is catalyzed by a hexokinase, which has a high affinity for glucose and is subjected to product inhibition by glucose-6-phosphate. Glucose taken up is immediately converted to glucose-6-phosphate. This allows a very low intracellular glucose concentration to be maintained that facilitates glucose transport in the direction of the glucose gradient. Until recently, hexokinase
was not considered to be a major site of acute regulation of glycolysis, and transport of glucose was considered to be the rate limiting step for glucose metabolism in skeletal muscle. However, it has been shown that insulin-sensitive tissues express a specific hexokinase, hexokinase II, the synthesis of which is controlled by insulin (25). Thus hexokinase II could be involved, in association with GLUT-4, in the long-term regulation of muscle glucose uptake.

Glucose-6-phosphate formed within cells is either converted to glycogen (see above) or rapidly converted to fructose-6-phosphate by a reversible non-rate-limiting enzymatic step catalyzed by phosphohexose isomerase. The next potential control point of glycolysis is the conversion of fructose-6-phosphate to fructose-1,6-diphosphate. This is catalyzed by phosphofructokinase, the activity of which is markedly increased by fructose-2,6-bisphosphate. The concentration of fructose-2,6-bisphosphate increases following glucose or insulin administration or after a meal and decreases after fasting and in experimental models of diabetes (13,14). The next steps involve the conversion of fructose-1,6-diphosphate to PEP by reactions that are not highly regulated. The last step of glycolysis, the conversion of PEP to pyruvate, is catalyzed by pyruvate kinase, which is highly regulated. Once pyruvate is formed, the redox state of the tissue and the activities of pyruvate dehydrogenase and pyruvate carboxylase determine the extent to which the pyruvate undergoes oxidation in mitochondria in the Krebs cycle or is reduced to lactate by lactate dehydrogenase.

Mechanisms Involved in the Control of Glucose Oxidation

Pyruvate, which is formed in the cytoplasm, is transported into the mitochondria and, if adequate NAD\(^+\) is available, is converted to acetyl-CoA and ultimately to CO\(_2\) and H\(_2\)O. The key step determining pyruvate flux through the Krebs cycle is the pyruvate dehydrogenase complex.

Insulin activates pyruvate dehydrogenase by dephosphorylation. In most tissues, there is competition between glucose and free fatty acids as oxidative fuels, and free fatty acids are the preferred substrate. The preferential utilization of free fatty acids can have a profound effect on glucose metabolism by way of a complex series of steps collectively referred to as the glucose–fatty acid cycle (26), a concept that has now received widespread experimental support and is generally accepted as operating in humans. According to this concept, the steps of which are illustrated in Fig. 6, a rise in free fatty acid oxidation increases the mitochondrial acetyl-CoA/CoA ratio and cytosolic citrate levels. The increased acetyl-CoA/CoA ratio inhibits pyruvate dehydrogenase, reducing pyruvate formation and hence its oxidation. The increased cytosolic citrate levels inhibit phosphofructokinase, which results in an increase in glucose-6-phosphate, an inhibitor of hexokinase. Reduced activity of hexokinase via an increase in intracellular glucose will then reduce glucose transport (uptake) by tissues. This mechanism has been invoked to explain the reciprocal changes observed in glucose and lipid metabolism in a variety of physiological and pathological conditions (26). Thus, during starvation and diabetes, when rates of fat oxidation are
increased, there is impaired tissue glucose uptake and oxidation. Reduced suppression of lipid oxidation after ingestion of a meal may impair glycogen formation in diabetes because of impaired glucose uptake. Furthermore, increases in lipid oxidation also promote increased gluconeogenesis. Thus, alterations in lipid metabolism can affect both the production and the utilization of glucose.

Control of Hepatic Glucose Metabolism

Under postabsorptive conditions it is not possible to elicit significant net glucose uptake by the liver or splanchnic bed with either hyperinsulinemia, hyperglycemia, or hypoglycaemia, when imposed independently (27). Net glucose uptake by the liver is greater during enteral glucose administration or infusion of glucose into the portal vein than during peripheral venous glucose infusion. It has been postulated that there is a "portal signal" evoked during enteral or intraportal glucose delivery that enhances glucose uptake by the liver. The nature of the "portal signal" is not yet known. Both humoral and neural factors must be considered. Gastrointestinal peptides (glucagon-like peptide 1, gastrin, neurotensin) released during glucose ingestion could be involved. These peptides are all insulino tropic and contribute to the improved glucose tolerance following glucose ingestion by promoting peripheral glucose removal and inhibiting hepatic glucose production (incretin effect), but there
are no data to suggest that they are directly involved in the enhanced hepatic glucose uptake. It appears that the enhanced glucose uptake by the liver in response to intraportal glucose delivery is not just the consequence of increased portal glucose levels but also involves the negative glucose gradient between arterial and portal venous blood. Because the ability of portal glucose delivery to enhance net hepatic glucose uptake is abolished by hepatic denervation (28), this suggests that the "portal signal" involves the autonomic nervous system. Whether this signal is generated locally or involves a comparison between portal venous glucose and the brain arterial glucose levels remains to be clarified. There is evidence for both afferent fibers in the hepatic branch of the vagus nerve and neurons in the lateral hypothalamus that can detect changes in glucose levels in the portal vein and efferent fibers that can alter net hepatic glucose uptake (29). Thus, a neural loop can serve to regulate hepatic glucose uptake.

**Mechanisms Involved in the Control of Hepatic Glucose Uptake**

The liver is considered to be "freely permeable to glucose" since GLUT-2, the principal glucose transporter expressed in this tissue, has a low apparent affinity for glucose (K_m = 20 mM). This allows equilibration between extracellular and intracellular glucose on a minute-to-minute basis. In this tissue, glucose phosphorylation by glucokinase, a specific hexokinase that has a low affinity for glucose (K_m = 10 mM) and is not inhibited by glucose-6-phosphate, is considered to be the rate-limiting step in overall glucose metabolism. Recently, the short-term regulation of hepatic glucokinase has been described (30). Glucokinase is inhibited by physiological levels of fructose-6-phosphate and this inhibition is released by fructose-1-phosphate. These effects are due to a modification of the affinity of glucokinase for glucose by means of a regulatory protein (31). The mechanism of action of this regulatory protein has recently been clarified (31). Fructose-6-phosphate binds to the regulatory protein and promotes the formation of a complex between glucokinase and the regulatory protein. Under these conditions, glucokinase is inactivated. Fructose-1-phosphate inhibits the formation of this complex and glucokinase is activated. The liver fructose-6-phosphate level is at least 10 μmol/liter in most physiological conditions. In the postabsorptive state, liver does not contain fructose-1-phosphate, and glucokinase is inhibited by the regulatory protein. After a meal that contains significant amounts of fructose, liver fructose-1-phosphate levels increase and the inhibition of glucokinase by the regulatory protein is released. Since fructose is present in most foods of vegetable origin, it can play a role as a signal to stimulate liver glucose utilization after a meal.

**Mechanisms Involved in the Control of Hepatic Glycogen Synthesis**

Following ingestion of a meal, hepatic glycogen stores are repleted via two metabolic pathways (32). In the first pathway, called the direct pathway, glucose is taken
up, phosphorylated to glucose-6-phosphate, transformed to glucose-1-phosphate, converted to UDP-glucose, and then incorporated into glycogen (Fig. 1). The last reaction is catalyzed by glycogen synthesis and is considered to be the rate-limiting step in that process. The molecular mechanisms by which glucose is able to increase liver glycogen synthesis involve the binding of glucose to phosphorylase a and its inactivation (Fig. 1). Since phosphorylase a is a potent inhibitor of synthase phosphatase, its inhibition allows the latter enzyme to activate glycogen synthase and to initiate glycogen synthesis after a lag period of a few minutes. The molecular mechanisms by which insulin increases liver glycogen synthesis are much less established. Insulin could activate protein phosphatase-1 and stimulate the dephosphorylation of glycogen synthase, leading to an increase of glycogen synthesis (Fig. 1).

The other pathway, called the indirect pathway, initially involves the metabolism of glucose to gluconeogenic precursors (mainly lactate and alanine), which are subsequently converted to glucose-6-phosphate in the liver and then to glycogen by steps identical to those involved in the direct pathway. The exact proportion of glycogen formed via each pathway and the source of the gluconeogenic substrates used for the indirect pathway have not been precisely determined. The relative proportions depend on species, antecedent diet, and duration of fast. In humans fasted overnight, it has been estimated that the direct pathway may account for 40–60% (33,34).

The significance of the direct versus indirect pathways and the source of substrates used for the indirect pathway lies in the potential influence of peripheral (extrahepatic) glucose metabolism on the liver. It was originally proposed that production of these precursors occurred in skeletal muscle (32). However, balance studies in dogs have shown that neither skeletal muscle, (35) the brain (36), nor the gastrointestinal tract (37) are major sources of lactate and alanine after an oral glucose load. This leaves the liver itself as the possible site for generation of the gluconeogenic precursors. The liver is indeed capable of producing lactate, while also taking it up and converting it to glycogen (37). The liver may be a major source of the gluconeogenic precursors used for the indirect pathway of glycogen synthesis. This can be explained by the fact that periportal hepatocytes are mainly gluconeogenic, whereas perivenous hepatocytes are mainly glycolytic (38). After administration of an oral glucose load, the perivenous hepatocytes take up glucose, synthesize glycogen, and release lactate into the circulation. Lactate is then transported to the periportal hepatocytes and converted to glycogen via gluconeogenesis (38).

REFERENCES

DISCUSSION

Dr. Schwartz: The data you show really refer to the adult, whether canine or human, and I would like to point out that the young infant has a hepatic output that is two or three times that of the adult, so where you showed a value of 1 mg/kg/min, the young infant requires 2–4 and maybe even 4–6 mg/kg/min. I should also like to point out something we all recognize which is that the infant has a very large brain; the mass of the brain is most important in terms of glucose uptake. Could you comment on the role of other substrates? What about ketones and lactate for example?

Dr. Girard: Hepatic glucose production is indeed two- to threefold higher in newborn babies than in the adult. In neonates, gluconeogenesis is a very important pathway—much more important after an overnight fast than in the adult—so regulation by insulin is less efficient. It has been clearly shown that hyperglycemia in babies may be due to a lack of effect of insulin on gluconeogenesis.

The second question is the use of alternate substrates by the brain and other tissues. The food intake of breast-fed babies in the first week of life is high in fat. When you calculate the amount of carbohydrate provided by the milk, and compare it with the rate of glucose utilization by the brain, the amount of glucose provided is at most 40–50% of that needed by the peripheral tissues. So gluconeogenesis is a very important pathway of glucose homeostasis at that time. The other aspect is that immediately after birth the liver is capable of converting free fatty acids to ketones to provide additional substrate for the brain, not only for use as a fuel but also as substrate for lipogenesis, for brain myelin formation. So it is true that alternative fuels are very important and that babies who have a defect of both glucose production and fatty acid oxidation, for example, those with a deficiency of the enzyme that allows the entry of long-chain fatty acids into the mitochondria, carnitine palmitoyl transferase-1, are in a very difficult situation, since they have both very low blood glucose and ketone body concentrations.

Dr. Drash: I want to extend Dr. Schwartz’s comments up the developmental scale a little to adolescence. Many things change during adolescence; in particular there is a state of insulin resistance. My colleague Dr. Silva Arslanian has been doing very elegant work, documenting major changes in energy flow as you go from pre-adolescence to adolescence (1,2). Adolescence is one of the few situations in which insulin resistance seems to be of positive value because it tends to push the system toward protein accretion. We think it is a major factor responsible for growth acceleration in adolescence. On the other hand, this is the time when we see the peak incidence of childhood diabetes. I think this may be at least partly related to this normal insulin resistance.

Dr. Girard: This is a very important point. I have no explanation for the appearance of insulin resistance at puberty. There is no evidence that the liver is involved in this process, but it is well known that there is a marked change in the mass of tissue, for example, both fat and protein accretion occur at this time. This is also the time when the sex hormones are secreted in increasing concentrations, and it is well known that some of these hormones have
anti-insulin effects on skeletal muscle and peripheral tissues. So the state of insulin resistance could be due to sexual maturation and the secretion of hormones at this time, and I feel that the effect is largely taking place in peripheral tissues rather than in the liver.

**Dr. Carrascosa:** During puberty there is a progressive increase in insulin growth factor-1 levels, and this means that there is an increasing availability of this growth factor, which as you know has an insulin-like effect, at the level of peripheral tissues. Do you think this increasing insulin growth factor level during puberty may play any role in the insulin resistance of diabetics in this period?

**Dr. Girard:** I don’t think so since insulin growth factor-1 has the same effect as insulin but at higher concentrations. If you infuse insulin growth factor-1 in humans, you can increase glucose utilization despite a state of insulin resistance. But if you are implying that insulin growth factor-1 is the result of oversecretion of growth hormone, then it should be pointed out that growth hormone has anti-insulin effects and these effects could be mediated through increased growth hormone secretion rather than through overproduction of insulin growth factor-1.

**Dr. Cowett:** What about the situation in very elderly people in whom there is a higher frequency of non-insulin-dependent diabetes? What is the possibility that there is altered enzyme sensitivity in the liver in elderly people?

**Dr. Girard:** In type 2 diabetes, hepatic glucose production after an overnight fast, is 50% higher than in normal people. In normal people, 50% of glucose production is derived from glycogen breakdown and 50% from gluconeogenesis. In type 2 diabetes, gluconeogenesis represents about 75% of hepatic glucose production. Perhaps what we describe as a state of liver insulin resistance could be due not only to a defect in transmission of the insulin message but also to the fact that the liver is using a different metabolic pathway for glucose production. I think it is very important to focus on this aspect. If you study patients with type 2 diabetes using the euglycemic clamp and you increase the insulin levels from 10 µunits/ml to 100 µunits/ml, what is clear is that you are capable of completely inhibiting hepatic glucose production whereas stimulation of glucose utilization is reduced only by 50%. This indicates that the liver is less sensitive to insulin but is not insulin resistant. My explanation is that the pathway used for producing glucose in type 2 diabetes is gluconeogenesis and that gluconeogenesis is less sensitive to insulin than glycogen breakdown.

**Dr. Nattrass:** Your illustrations tend to suggest that the endpoint of gluconeogenesis is glucose, although in one of your later figures it appears that glycogen may also be an endpoint. I wonder what effect this has upon the interpretation of, say, the effect of insulin on gluconeogenesis when you measure alanine and lactate uptake into glucose rather than into glycogen.

I should also like to emphasize the importance of the differential effects of insulin upon metabolism. I think this is very underappreciated by clinicians. For example, inhibition of lipolysis takes very little insulin, whereas stimulation of glucose uptake into cells takes a good deal.

**Dr. Girard:** Your question concerns what we call the glucose paradox. This is the situation where, between starvation and refeeding, part of the glycogen repletion in the liver is achieved not only by hepatic uptake of glucose but also by gluconeogenesis which, instead of providing glucose for the blood, provides glycogen for repletion. This was an active field of research about 5–6 years ago. It seems to me from reading the original publication (3) that if you consume a large amount of carbohydrate, the direct pathway (that is, the conversion of glucose to glycogen) should be the most important. But in a situation where you provide a mixed meal, where there is less glucose in the meal and also substrates for gluconeogenesis, then gluconeogenesis is partly used to replenish glycogen stores in the liver, up to a maximum
of about 50%. These studies have mainly been done in rodents, and I am not sure the results can necessarily be extrapolated to humans.

Dr. Bergman: I agree with Dr. Girard. I think the issue that was being addressed is the question of whether it is necessary to suppress the gluconeogenic pathway in order to suppress glucose production from non-carbohydrate precursors. I believe there is evidence that hormonal control, rather than suppressing gluconeogenesis, actually suppresses the ultimate phase of carbohydrate metabolism as it goes up the pathway, whether it goes toward glycogen or toward glucose. It is the direction of the final glucose pathway, whether it goes toward glycogen or toward glucose in the blood, that may be under control rather than the pathway itself.

I should also like to add a comment following Dr. Nattrass’s remarks (above) on the differential sensitivity of various tissues to insulin. One of the things that has impressed us recently is the fact that the concentration of insulin at the cells may be quite different from its concentration in the blood. In the liver, the hepatocytes are exposed to more or less the same concentration of insulin as in the portal blood because of good transfer characteristics across the capillary endothelium. On the other hand, in muscle, which has a very tight capillary endothelium and inefficient insulin transfer, the concentration of insulin at the muscle cell is much lower than it is in the blood. When we interpret the sensitivity of these tissues to insulin we need to think in terms not only of the blood insulin but of insulin in the lymph.

Dr. Marliss: My question relates to the stimulation of hepatic glycogenolysis in humans by catecholamines. Are you going to stand firmly by the premise that this is \( \beta \) receptor mediated or will you accept the possibility that this is not absolutely clear as yet?

Dr. Girard: From the work of Riza and De Fronzo (4,5) it is clear that, in the resting state, a large part of glycogenolysis is due to a \( \beta \)-adrenergic mechanism in the human. This conclusion is mainly based on the fact that if you use a \( \beta \)-adrenergic inhibitor you can completely suppress the effect of catecholamines. It is possible, however, that what occurs during the physiological release of catecholamines could be markedly different since it seems that norepinephrine and epinephrine are not acting in the same way or on the same metabolic pathways. From the work Cherrington (6), it seems that norepinephrine is a good stimulator of gluconeogenesis but not of glycogen breakdown. However, there is much controversy about this since the proportion of the catecholamine effect that is due to the activation of \( \beta \)-adrenergic or \( \alpha \)-adrenergic receptors varies among the species. In the rodent, most of the effect of catecholamines acts through \( \alpha \)-adrenergic receptors. In the human, it seems that much of the effect is mediated through \( \beta \)-adrenergic receptors, as in the dog.

Dr. Crofford: Another problem in interpreting these data is that most of the experiments performed in humans, and indeed most of the ones performed by Cherrington in dogs, were performed in the resting state; the effect of exercise, however, is dramatic in terms of the disposition of a particular meal or glucose load, and I think that should be taken into consideration when one interprets the fraction of the nutrient that is distributed and taken up by the various tissues.

Dr. Girard: It has been shown that, when exercise is stopped and glycogen stores are repleted, there is a predominance of skeletal muscle glycogen restoration over hepatic glycogen restoration. So I agree that we have to take into account that what I have described refers to the resting state.

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