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REGULATION OF GLUCOSE PRODUCTION BY THE LIVER

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ABSTRACT

Glucose is an essential nutrient for the human body. It is the major energy source for many cells, which depend on the bloodstream for a steady supply. Blood glucose levels, therefore, are carefully maintained. The liver plays a central role in this process by balancing the uptake and storage of glucose via glycogenesis and the release of glucose via glycogenolysis and gluconeogenesis. The several substrate cycles in the major metabolic pathways of the liver play key roles in the regulation of glucose production. In this review, we focus on the short- and long-term regulation glucose-6-phosphatase and its substrate cycle counterpart, glucokinase. The substrate cycle enzyme glucose-6-phosphatase catalyzes the terminal step in both the gluconeogenic and glycogenolytic pathways and is opposed by the glycolytic enzyme glucokinase. In addition, we include the regulation of GLUT 2, which facilitates the final step in the transport of glucose out of the liver and into the bloodstream.

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INTRODUCTION

Glucose is the major energy source for many mammalian cells. These cells depend on a steady supply of glucose, much of which is provided through the bloodstream. Therefore, blood glucose levels are carefully maintained. This maintenance is achieved through a balance of several factors, including the rate of consumption and intestinal absorption of dietary carbohydrate, the rate of utilization of glucose by peripheral tissues and the loss of glucose through the kidney tubule, and the rate of removal or release of glucose by the liver (75).

The liver plays a major role in blood glucose homeostasis by maintaining a balance between the uptake and storage of glucose via glycogenesis and the release of glucose via glycogenolysis and gluconeogenesis. Maintaining blood glucose levels within a narrow range requires the regulation of two major metabolic pathways, gluconeogenesis and glycogenolysis, which produce glucose in the liver. In addition, key enzymes in opposing metabolic pathways, glycolysis and glycogenesis, respectively, must also be regulated in order for net flux in the appropriate direction to be achieved. Several regulatory enzymes play key roles in this process and are part of substrate cycles in which cycling between the substrates and products of the enzymes occurs (see Figure 1). Two enzymes specific for gluconeogenesis, phosphoenolpyruvate carboxykinase (PEPCK) and fructose-1,6-bisphosphatase (Fru-1,6-P_2ase), are opposed by the glycolytic enzymes pyruvate kinase (PK) and 6-phosphofructo-1-kinase (PFK). The glycogenolytic-specific enzyme glycogen phosphorylase is opposed by the glycogenic enzyme glycogen synthase. The enzyme glucose-6-phosphatase (Glc-6-Pase) catalyzes the terminal step in both the gluconeogenic and glycogenolytic pathways and is opposed by the glycolytic enzyme glucokinase (GK). These metabolic cycles provide a system in which the rate and direction of flux can be finely regulated by small changes in the concentration

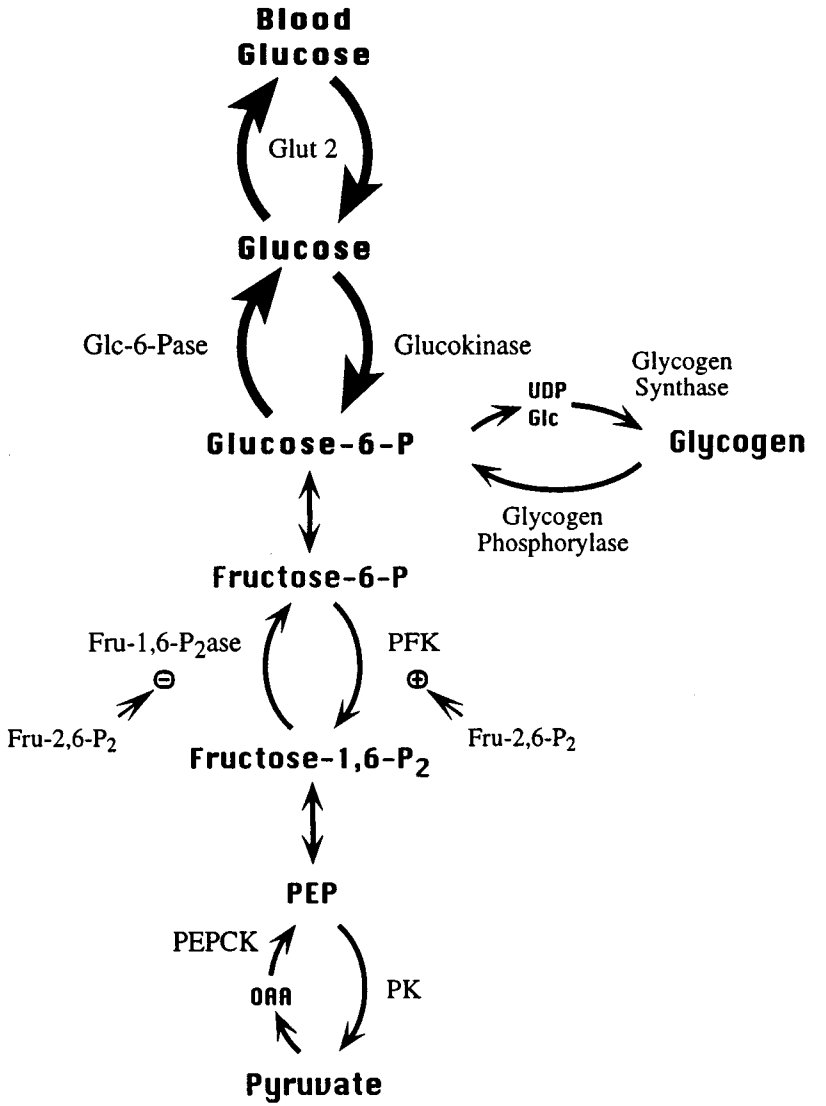

Figure 1 Substrate cycles in the glycolytic/gluconeogenic and glycogenic/glycogenolytic pathways that are involved in the regulation of glucose production by the liver. Fructose-2,6-bisphosphate (Fru-2,6-P_2) is an activator of phosphofructo-1-kinase (PFK) and an inhibitor of Fru-1,6-P_2ase. GLUT 2, hepatic glucose transporter; Glc-6-Pase, glucose-6-phosphatase; UDP Glc, uridine diphosphate glucose; PEP, phosphoenolpyruvate; PEPCK, phosphoenolpyruvate carboxykinase; OAA, oxaloacetate; PK, pyruvate kinase.

of specific effectors of one or more of the substrate cycle enzymes and/or by covalent modification of many of these enzymes. In addition, metabolic flux can be influenced by changes in the gene expression of these substrate cycle enzymes.

In addition to the substrate cycle enzymes, the bifunctional enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (6-PF-2-K/Fru-2,6-P_2ase) plays a unique role in the regulation of gluconeogenesis and glycolysis in the liver. The regulatory molecule, fructose 2,6-bisphosphate (fru-2,6-P_2), synthesized by this enzyme is a potent allosteric activator of PFK and inhibitor of Fru-1,6-P_2ase in the liver. Both the synthesis and degradation of fru-2,6-P_2 are catalyzed by this bifunctional enzyme. The kinase and bisphosphatase activities of the enzyme are reciprocally regulated by cAMP-dependent protein kinase-catalyzed phosphorylation. The modulation of fru-2,6-P_2 levels by this regulatory enzyme is thought to be a metabolic switching mechanism between gluconeogenesis and glycolysis (87).

The regulation of gluconeogenesis/glycolysis and glycogenolysis/glycogenesis at the level of the substrate cycle enzymes (34, 39, 58, 86, 87) and 6-PF-2-K/Fru-2,6-P_2ase (85, 87, 88, 106) has been reviewed extensively in recent years, with one notable exception. Until now, little was known about the regulation of the enzyme Glc-6-Pase. The recent cloning and the discovery of potential metabolic inhibitors of the enzyme have made it possible to examine its regulation both acutely and at the level of gene expression. Because of its critical position as the terminal step in both the glycogenolytic and gluconeogenic pathways, Glc-6-Pase is a key determinant in the production of glucose by the liver.

In this review, we focus on the short- and long-term regulation of Glc-6-Pase and its substrate cycle counterpart, GK (see Figure 1). A shifting between glucose phosphorylation and glucose-6-phosphate (glc-6-P) hydrolysis in the liver is achieved by the differential regulation of these two enzymes, which directly affects hepatic glucose production. In addition, we discuss the regulation of GLUT 2, which facilitates the terminal step in the transport of glucose out of the liver and into the bloodstream. A number of subcellular components are involved in this multistep process and are illustrated in Figure 2.

GLUCOSE-6-PHOSPHATASE

Multiple Activities and Components of the Glucose-6-Phosphatase System

It has been established over the years that Glc-6-Pase is a multifunctional enzyme (69, 75). The classical function of Glc-6-Pase is the hydrolysis of glc-6-P

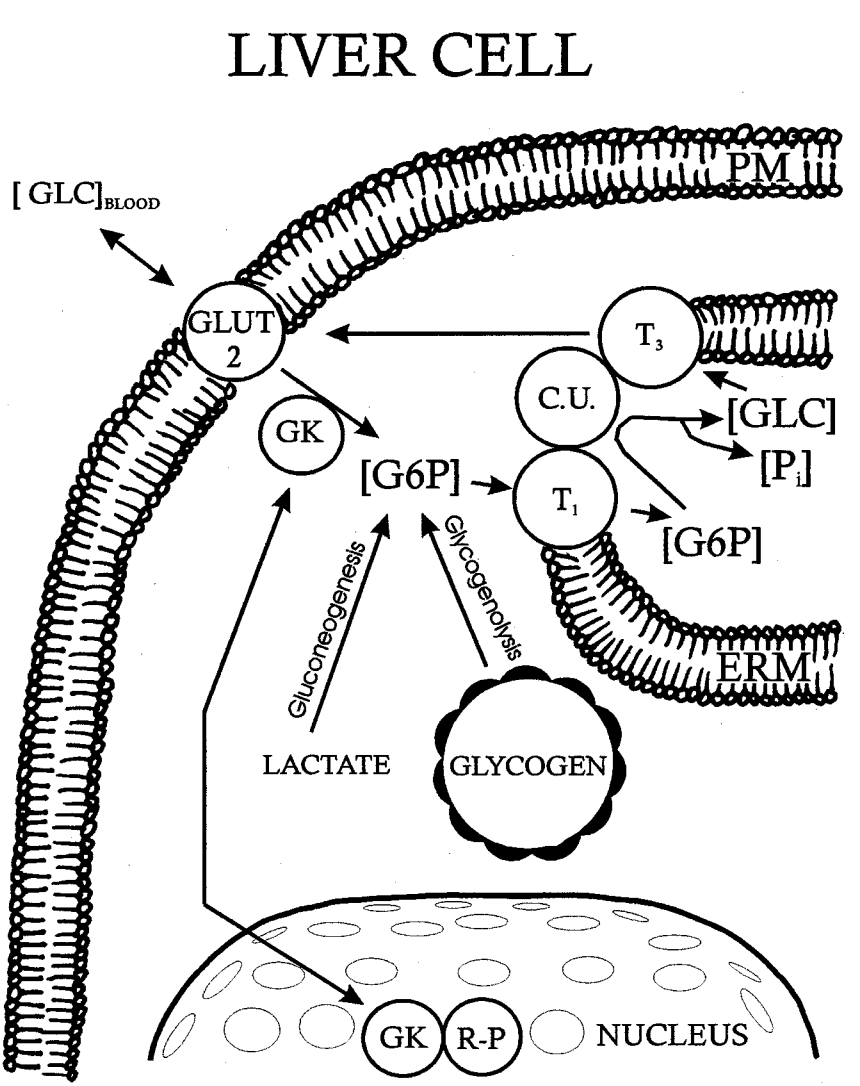

Figure 2 A schematic representation of the terminal steps in the production of glucose by the liver. GLUT 2, hepatic glucose transporter; GK, glucokinase; R-P, regulatory protein; C.U., Glc-6-Pase catalytic unit; T_1, glc-6-P transporter; T_3, glucose transporter; GLC, glucose; G6P, glucose-6-phosphate; P_i, inorganic phosphate; ERM, endoplasmic reticulum membrane; PM, plasma membrane.

(Reaction 1). The enzyme also catalyzes the hydrolysis of inorganic pyrophosphate (PP_i) (Reaction 2). In addition, the enzyme is capable of glc-6-P synthesis via potent phosphotransferase activity (72, 70) where carbamyl-P or PP_i serve as phosphoryl donors (Reactions 3 and 4, respectively). The V_{max} of the biosynthetic activity may equal or actually exceed that of the enzyme's hydrolytic capacity (51) and provides an alternative to insulin-dependent GK for hepatic glucose phosphorylation (70, 75).

$$\text{glc-6-P} + H_2O \rightarrow \text{glucose} + P_i \quad 1.$$

$$PP_i + H_2O \rightarrow 2P_i \quad 2.$$

$$\text{Carbamyl-P} + \text{glucose} \rightarrow \text{glc-6-P} + NH_3 + CO_2 \quad 3.$$

$$PP_i + \text{glucose} \rightarrow \text{glc-6-P} + P_i \quad 4.$$

Glc-6-Pase was once thought to be a single enzyme protein, but a multitude of studies (7, 8, 23, 25, 79, 99) over the past 30 years have led to the view that Glc-6-Pase is a multicomponent system. Currently, two hypotheses of structure-function relationship exist for the enzyme, the "substrate transport-catalytic unit" hypothesis (6, 7, 8) and the "combined conformational flexibility-substrate transport" hypothesis (15, 93). The former hypothesis proposes that the catalytic unit of Glc-6-Pase is sequestered on the luminal side of the endoplasmic reticulum (ER), and at least four additional membrane-spanning translocases allow substrates access to the catalytically active site. The latter hypothesis proposes that the enzyme is deeply embedded within the ER membrane, and this single protein possesses both catalytic and substrate/product transport activities. However, proponents of this latter hypothesis acknowledge the likely existence of additional auxiliary proteins, which may effect catalytic unit activity.

The multicomponent nature of the Glc-6-Pase system provides several sites at which regulation may occur. For example, an effector may interact at one of the specific translocases or auxiliary proteins, producing a specific effect on either the hydrolytic or biosynthetic activity, whereas another effector may interact with the catalytic unit of the system, producing a general effect on all activities of the enzyme. In addition, each component of the system may be regulated in terms of gene expression. The substrate transport-catalytic unit hypothesis is most widely accepted and therefore we limit our discussion on the regulation of this enzyme in this review to this hypothesis. However, it is likely that aspects of both hypotheses will in time become integrated into a single comprehensive hypothesis.

Acute Regulation of Glucose-6-Phosphatase

Some progress has been made in recent years on the acute regulation of the Glc-6-Pase system. Various metabolites of carbohydrate, lipid, and protein catabolism have been identified as inhibitors of glc-6-P hydrolysis. In addition, products of phosphatidylinositol (PI) kinase activity have also been identified as inhibitors of glc-6-P hydrolysis, which may be particularly important as a result of PI kinase involvement in various signal transduction pathways. Also, a mechanism by which inhibition by chloride ion of the synthetic activity of Glc-6-Pase is reversed while the hydrolytic activity remains inhibited has recently been reported.

Details regarding the various mechanisms by which Glc-6-Pase may be regulated are expanded on in the remainder of this section.

FRUCTOSE-1-P Studies indicate that fructose-1-P may play a regulatory role by directing glc-6-P away from glucose formation and toward glycogen synthesis and glycolysis (91). Fructose-1-P inhibited glc-6-P hydrolysis but the amount of inhibition calculated with near-physiologic concentrations of inhibitor and substrate were small. However, when hepatic fructose-1-P levels are elevated in response to a dietary fructose load (91, 104, 116), an increase in inhibition with intact microsomes from a basal value of approximately 1% to an elevated level of 31% is predicted (91). These observations suggest the potential for metabolic regulation of Glc-6-Pase hydrolytic activity by fructose-1-P. Inhibition of glc-6-P hydrolysis by fructose-1-P would result in an increase in hepatic glc-6-P concentration and would direct glc-6-P away from glucose production and toward glycogen synthesis and glycolysis. In addition, a fructose-1-P–associated activation of GK (107, see below) fits well with this hypothesis. The reciprocal effects of fructose-1-P on Glc-6-Pase and GK may explain why blood glucose level rises only modestly following a fructose load (44) and may rationalize the synergism between glucose and fructose in regard to glycogenesis (117).

A PROLINE METABOLITE A mechanism involving a simple increase in osmolarity (13) has been proposed to explain amino acid–induced stimulation of glycogenesis from glucose and gluconeogenic substrates. However, this mechanism does not account for L-proline's stimulatory effect (13). Enhanced glycogenesis from glucose in the presence of 10 mM L-proline is greater than can be explained on the basis of an osmotically induced increase in hepatocyte volume (13, 55). Studies in which the production of glucose and glycogen from L-proline and L-glutamine was measured in isolated perfused livers from rats fasted for 48 h revealed that both amino acids were gluconeogenic but only L-proline was glycogenic (16). Although both amino acids are gluconeogenic through a common α-ketoglutarate pathway, a marked increase in

hepatic glc-6-P concentration in the presence of L-proline but not L-glutamine was observed (16). This indicated that a proline metabolite inhibits glc-6-P hydrolysis and directs glc-6-P away from glucose production and toward glycogenesis. The inhibitory metabolite has not been identified. However, the proline metabolites hydroxyproline and δ^1-pyrrolidine-5-carboxylate as well as proline itself have been eliminated as possibilities (16, 79).

α-KETOGLUTARATE Mithieux and coworkers reported in 1990 that α-ketoglutarate (α-KG) in the presence of Mg^{2+} ($Mg^{2+} \cdot \alpha$-KG) inhibited hepatic glc-6-P hydrolysis catalyzed by Glc-6-Pase in intact microsomes (63). Disruption of microsomal integrity by detergents abolished the $Mg^{2+} \cdot \alpha$-KG inhibitory effect, which suggested that the inhibitory event was manifest at the glc-6-P translocase component of the Glc-6-Pase system. More recently, Minassian et al suggested that $Mg^{2+} \cdot \alpha$-KG may be involved in metabolite inhibition of glc-6-P hydrolysis by Glc-6-Pase and that it may constitute the mechanism underlying enhanced glycogenesis rebound in the liver after 72 h of fasting (56). An increase in hepatic glc-6-P concentration in rats fasted for 72 h over that of rats fasted for 48 h indicated that hydrolysis of glc-6-P by Glc-6-Pase was diminished. A corresponding 2.5- to 3-fold increase in hepatic α-KG concentration in rats fasted for 72 h over those fasted for 48 h suggested that glc-6-P hydrolysis may be inhibited by approximately 25%–30% by $Mg^{2+} \cdot \alpha$-KG, resulting in a rebound in hepatic glycogen (56).

FATTY ACIDS Both saturated and unsaturated fatty-acyl–coenzyme A (CoA) compounds inhibit hepatic Glc-6-Pase (36, 64, 80). However, only unsaturated fatty acids such as arachidonate inhibit the enzyme; saturated fatty acids do not (31, 35, 59, 60). Unsaturated fatty acids inhibit Glc-6-Pase of untreated microsomes to the same extent as that from detergent-disrupted microsomes. In contrast, inhibition by fatty-acyl–CoA compounds is much higher with untreated than with detergent-treated microsomes, which suggests that the glc-6-P translocase of the system is a site of inhibition (35, 64). In addition, the fatty-acyl–CoA–derived inhibitory effect requires a minimum chain length of 10 carbons and becomes more potent with increasing chain length (64). Long-chain fatty-acyl–CoA compounds ($\geq$16 carbons) inhibit glc-6-P hydrolysis catalyzed by untreated microsomes within the range of 1–2 μM, but when higher concentrations were utilized the inhibitory effect was either partially or totally reversed or resulted in an activation (64, 80). This activation is due to detergent effects of these compounds when used at higher concentrations (64, 80). It is interesting to note that GK is also inhibited by long-chain fatty-acyl–CoA compounds within this micromolar range ($K_i = 1.8$ μM) (101).

Because both unsaturated fatty acids and fatty-acyl–CoA compounds inhibit Glc-6-Pase at very low concentrations and the enzyme is located within a

lipid-rich environment, it is possible that Glc-6-Pase is regulated by these compounds. In addition, Danièle et al recently identified unsaturated fatty acids (31) as the previously reported (50) glycogen-associated inhibitor of hepatic Glc-6-Pase. Because glycogen is associated with ER membranes in the liver (26), the presence of unsaturated fatty acids within the glycogen granule may also play an important role in the regulation of Glc-6-Pase.

The actual in vivo concentrations of these inhibitory fatty acids within the ER and changes in their levels have not been measured and are experimentally difficult to accurately determine (35, 59). However, it is reasonable to assume that ER fatty acid levels and their ability to inhibit Glc-6-Pase will fluctuate under various nutritional and disease states (35, 59, 64).

PHOSPHOINOSITIDES Glc-6-Pase is inhibited in a dose-dependent manner by phosphoinositides within a concentration range of 0.5–10.0 μM (61). PI-3,4,5-P_3 is the most potent phosphoinositide inhibitor of Glc-6-Pase (K_i = 1.7 μM), followed by PI-3,4-P_2 and PI-4,5-P_2. PI-3,4-P_2, PI-4,5-P_2, and PI-3,4,5-P_3 are competitive inhibitors of the enzyme in both untreated and detergent-treated microsomes. However, in detergent-treated microsomes, Glc-6-Pase is less sensitive to inhibition by these compounds, and they are all equally effective as inhibitors. This suggests that sites in addition to the catalytic unit of the system (e.g. the glc-6-P translocase) may be involved in the inhibitory effect.

The presence of PI kinases (84, 115) and their products (37) in the ER is consistent with their possible involvement in the acute regulation of Glc-6-Pase (61). The involvement of PI 3-kinase in insulin signaling (96), which may involve the localization of active PI 3-kinase to the ER in response to insulin (62, 84), suggests an interesting mechanism, in which glc-6-P hydrolysis catalyzed by Glc-6-Pase may be inhibited in response to insulin. This possible mechanism may explain the acute inhibitory effect on Glc-6-Pase activity observed in rats shortly after being refed following a 48-h fast (57, 62).

CHLORIDE ION INHIBITION AND ITS REVERSAL All the regulators of Glc-6-Pase above are described in terms of their inhibitory effects on the glc-6-P phosphohydrolase activity of the enzyme. Because of the multicomponent nature of the Glc-6-Pase system, it is possible to differentially regulate both the hydrolytic and synthetic activities of the enzyme. Recently, this type of regulation was described with Glc-6-Pase, and it was proposed that the biosynthetic activity of the enzyme may act in concert with glycogen synthase during amino acid–induced glycogenesis from glucose (83).

Chloride ion competitively inhibits both the synthetic and hydrolytic activities of Glc-6-Pase (83). However, with undisrupted microsomes, chloride ion inhibits the carbamyl phosphate—glucose phosphotransferase (K_i = 19 mM)

activity more extensively than the glc-6-P phosphohydrolase (K_i = 117 mM) activity of the enzyme.

A "regulatory volume-decrease mechanism" explaining amino acid–stimulated glycogenesis from glucose has been defined (55). It involves amino acid–induced swelling of hepatocytes resulting in the loss of chloride ions (from approximately 35 to 15 mM), which leads to lessened inhibition of glycogen synthase phosphatase. This results in enhanced conversion of the inactive to the active form of glycogen synthase and, thus, enhanced glycogen synthesis. In addition, reduced chloride ion concentration will result in lessened inhibition of the biosynthetic activity of Glc-6-Pase and have little effect on the hydrolytic activity (note the large difference in K_i values for chloride between the hydrolytic and synthetic activities of Glc-6-Pase above). This will lead to an increase in cellular concentration of glc-6-P, an important glycogenic intermediate as well as allosteric activator of glycogen synthase. The net result is an increase in glycogen synthesis in response to amino acids.

Regulation of Hepatic Glucose-6-Phosphatase Gene Expression

Until recently, little was known regarding the gene expression of the Glc-6-Pase catalytic unit. Over the years, a number of inferences about Glc-6-Pase gene expression have been drawn from several studies in which differences in the activity of the enzyme were measured under various nutritional and hormonal states. Liver microsomal Glc-6-Pase activity increases with fasting and in the diabetic state (9, 76, 78, 94). The administration of insulin to diabetic rats results in the decrease of Glc-6-Pase activity levels (10, 76, 78). In addition, glucocorticoids have been shown to increase hepatic Glc-6-Pase activity levels in adrenalectomized rats (77).

The development of cDNA probes for the catalytic unit of the enzyme (45, 48) and the identification of promotor regions in the Glc-6-Pase gene (4, 92, 98) have recently made it possible to explore the mechanisms at the level of gene expression behind the changes in activity observed under various nutritional and hormonal states.

GLUCOCORTICOIDS AND cAMP In Fao cells, cAMP increased Glc-6-Pase mRNA fourfold after 3 h, but a longer exposure (24 h) to cAMP decreased mRNA levels to 50% of control values (4, 45). Treatment of Fao cells with the glucocorticoid dexamethasone resulted in a relatively slow response, with a 3.3-fold increase in Glc-6-Pase mRNA levels after 48 h (4). In primary hepatocytes, however, the stimulatory effect of dexamethasone or cAMP is seen only when both hormones are added together (4). In addition, glucocorticoid (dexamethasone) treatment of adrenalectomized rats did not affect Glc-6-Pase mRNA levels (4).

The construction of a luciferase reporter gene plasmid containing the 5′ region of the human Glc-6-Pase gene and the subsequent transfection of H4IIE cells with this reporter plasmid (92) demonstrated the presence of hormonal response elements, because regulation was observed after 24 h in the presence of dexamethasone, dibutyryl-cAMP, or insulin. Dexamethasone increased luciferase activity in transfected cells by tenfold. In contrast, dibutyl cAMP alone did not significantly effect the luciferase activity but had a significant effect when used in conjunction with dexamethasone (92). This effect was marked by a reduction in the stimulatory action of dexamethasone from a ten- to a threefold increase in luciferase activity over basal levels after 24 h of treatment with these compounds.

Because the effects of glucocorticoids and cAMP on Glc-6-Pase gene expression in terms of relative mRNA levels are not consistent and are dependent on time and the cellular model utilized, it will be more difficult to determine the mechanisms of their effects. It is clear, however, that the effect of glucocorticoids may be dependent on other hormones, such as glucagon (via cAMP) and insulin.

INSULIN Insulin has a dominant negative effect on glucocorticoid stimulation of Glc-6-Pase mRNA levels (4). Both mRNA levels and activity of Glc-6-Pase are low in the fed and refed states, where insulin levels are elevated (4, 49, 53). Both mRNA levels and activity of Glc-6-Pase are elevated in diabetic rats (4), and administration of insulin to diabetic rats results in the reduction of Glc-6-Pase mRNA and activity (4). A multicomponent insulin response sequence (IRS) that mediates the strong repression by insulin has been identified in the proximal 5′ flanking region of the murine Glc-6-Pase gene (98). Deletion of the murine Glc-6-Pase sequence between bases −198 and −159 completely abolished the inhibitory insulin response when mouse Glc-6-Pase–chloramphenicol acetyltransferase (CAT) fusion genes were transiently expressed in hepatoma cells. This region contains three copies of the T(G/A)TTTTG sequence, which is the core motif of the PEPCK gene IRS (98). Recently, PI 3-kinase was shown to play a central role in the signaling pathway by which insulin mediates the repression of Glc-6-Pase transcription in H4IIE cells (32). In this study (32), wortmannin, a PI 3-kinase inhibitor, blocked the repression of Glc-6-Pase mRNA expression by insulin, and this repression was mimicked by the overexpression of a constitutively active PI 3-kinase.

GLUCOSE The observation of increased Glc-6-Pase mRNA in Fao cells incubated in the presence of high (25 mM) glucose first suggested that Glc-6-Pase gene expression may be regulated by glucose (45). Massillon et al recently presented data obtained from studies with 90% partially pancreatectomized rats that suggest the in vivo gene expression of Glc-6-Pase is regulated by glucose

independently from insulin (53). The relative abundance of Glc-6-Pase mRNA and protein is increased in the diabetic state and is normalized when plasma glucose concentration is decreased in the absence of insulin (53). The stimulatory effect of glucose on Glc-6-Pase mRNA has also been demonstrated in primary hepatocytes (3). The maximal stimulatory response at glucose concentrations as low as 11 mM was seen despite the presence of 1 μM insulin. In addition, overexpression of GK in Fao cells with high levels of glucose increased Glc-6-Pase mRNA levels 21-fold, which was correlated with an increase in lactate production (3). Under the same conditions, however, overexpression of HK I did not significantly increase lactate production and did not affect the level of Glc-6-Pase mRNA (3). These findings suggested that the hepatic gene expression of Glc-6-Pase by glucose is mediated by increased glucose metabolism via glycolysis. Massillon et al recently proposed that xylulose-5-P produced via carbon flux through the pentose phosphate pathway regulates the hepatic gene expression of Glc-6-Pase (54). In these studies, the inhibition of GK activity by glucosamine infusion into conscious rats blunted the stimulation of Glc-6-Pase gene expression by glucose, which suggested that a metabolite generated by the metabolism of glucose at or beyond glc-6-P was responsible for the regulatory effect of glucose. Furthermore, the infusion of xylitol, which is directly converted to xylulose-5-P, replicated the effect of hyperglycemia on Glc-6-Pase mRNA levels while not altering the hepatic concentrations of glc-6-P or fru-2,6-P_2 (54). The regulation of Glc-6-Pase gene expression by high levels of glucose is unexpected because glucose is a product of the hydrolytic activity of Glc-6-Pase, and this regulatory effect is, therefore, considered paradoxical.

FRUCTOSE-2,6-BISPHOSPHATE In the absence of hormonal treatment, the alteration of glycolytic and gluconeogenic flux via adenovirus vector overexpression of wild-type or double-mutant 6-PF-2-K/Fru-2,6-P_2ase has a marked effect on Glc-6-Pase mRNA levels in Fao cells (3). Overexpression of double-mutant 6-PF-2-K/Fru-2,6-P_2ase, which possesses only kinase activity and cannot be down-regulated by cAMP phosphorylation, resulted in a 15-fold increase in fru-2,6-P_2, with a concomitant increase in glycolytic flux, and led to a six-fold increase in Glc-6-Pase mRNA levels in Fao cells (3). Overexpression of wild-type 6-PF-2-K/Fru-2,6-P_2ase decreased fru-2,6-P_2 concentration by 70% relative to the control cells and also decreased Glc-6-Pase mRNA levels in Fao cells (3). These studies demonstrated the dependence of Glc-6-Pase gene expression on glycolytic flux without the addition of high levels of glucose. High levels of fru-2,6-P_2, which stimulates glycolytic flux, increase Glc-6-Pase gene expression, whereas low levels of fru-2,6-P_2, which stimulates gluconeogenic flux, reduce Glc-6-Pase gene expression. The underlying mechanism behind

the fru-2,6-P_2 stimulatory effect needs to be determined, but fru-2,6-P_2 itself and a glycolytically derived metabolite have been suggested as potential regulators (3). Xylulose-5-P, which is thought to be involved in the glucose stimulatory effect, is not thought to be the signaling molecule in this case because of the differential effects of fru-2,6-P_2 and glucose on L-PK gene expression (3). The stimulatory effect of high fru-2,6-P_2 on Glc-6-Pase gene expression is considered paradoxical or counter-regulatory because Glc-6-Pase is a key gluconeogenic enzyme and fru-2,6-P_2 is a potent inhibitor of Fru-1,6-P_2ase (3, 85, 88), another gluconeogenic-specific enzyme.

Metabolic Effects of Overexpression of Glucose-6-Phosphatase Catalytic Subunit

Because of their role in the production of glucose from the liver, the genes that code for the proteins of the Glc-6-Pase system have been thought to be candidate genes for susceptibility to non–insulin-dependent diabetes mellitus (NIDDM). The metabolic impact of overexpression of the gene coding for the catalytic subunit of Glc-6-Pase in liver has been demonstrated using gene delivery via a Glc-6-Pase catalytic subunit adenovirus vector (AdCMV-Glc-6-Pase) in both primary hepatocytes and in vivo infusion into rats (95, 102).

Relative to control cells, AdCMV-Glc-6-Pase–treated liver cells contain significantly less glycogen and glc-6-P but unchanged UDP-glucose levels. Furthermore, the glycogen synthase activity state was closely correlated with glc-6-P levels over a wide range of glucose concentrations in both Glc-6-Pase–overexpressing and control cells. The reduction in glycogen synthesis in AdCMV-Glc-6-Pase–treated hepatocytes is therefore not a function of decreased substrate availability but rather occurs because of the regulatory effects of glc-6-P on glycogen synthase activity. It was also found that AdCMV-Glc-6-Pase–treated cells had significantly lower rates of lactate production and [3-^{3}H]glucose usage, coupled with enhanced rates of gluconeogenesis and glc-6-P hydrolysis (95).

In AdCMV-Glc-6-Pase–infused normal rats, Glc-6-Pase enzymatic activity was increased by 67% compared with control infused rats (AdCMV-ßGalactosidase). In animals fasted for 48 h and refed overnight, plasma glucose levels were elevated in those infused with AdCMV-Glc-6-Pase compared with the control group (183 vs 148 mg/dl). An oral glucose tolerance test applied to rats fasted for 48 h also revealed a significantly higher and more prolonged hyperglycemic excursion in AdCMV-Glc-6-Pase–treated animals (glucose levels 120 min after glucose bolus, 241 vs 159 mg/dl). At the 120-min time point, insulin levels were increased by 74% in the AdCMV-Glc-6-Pase group, and plasma-free fatty acids and triglycerides were reduced by 55% and 75%, respectively. Furthermore, hepatocytes treated with AdCMV-Glc-6-Pase exhibit

a metabolic profile resembling that of liver cells from patients (e.g. an increase in Glc-6-Pase gene expression and activity) with NIDDM, which suggests that impaired regulation of the catalytic subunit of Glc-6-Pase could contribute to the etiology of the disease (102).

These studies clearly show that overexpression of the Glc-6-Pase catalytic subunit alone is sufficient to activate flux through the Glc-6-Pase system in liver cells, in vitro and in vivo. This also implies that the glc-6-P transporter may not limit the hydrolysis of glc-6-P (7, 8, 36). Furthermore, modest overexpression of the catalytic subunit of Glc-6-Pase in both these systems revealed metabolite profiles characteristic of early stage NIDDM. However, the hypolipemic profile seen in this model is markedly different from the hyperlipidemia characteristic of NIDDM.

Glucose-6-Phosphate Transport and Its Regulation

The glc-6-P transporter (T_1) has long been postulated to be an integral component of the Glc-6-Pase system. The transport hypothesis put forward by Arion and coworkers postulated that glc-6-P was transported into the lumen of the ER to the catalytic subunit of Glc-6-Pase by a distinct protein and that the catalytic subunit per se had no intrinsic transport activity (8). Direct evidence for the glc-6-P translocase has come from demonstration of glc-6-P uptake into microsomes using centrifugation through silicone oil to provide rapid quenching, filtration, and light scattering techniques (11, 12, 65). The strongest evidence for the existence of such a transporter comes from genetic studies on glycogen storage disease (GSD), where type 1b GSD was associated with a transport defect in glc-6-P transport (46) and type 1b GSD patients were found to be normal for the Glc-6-Pase catalytic subunit (47). Recently, a cDNA clone has been isolated from human liver coding for the putative microsomal glc-6-P transporter (36). This sequence was elucidated using mouse ESTs with homology to a bacterial transport protein. Functional activity has yet to be demonstrated; however, mutations in the putative glc-6-P transporter coding region were found in two GSD type 1b patients.

Although information on the regulation of the gene expression of the glc-6-P transporter gene must await isolation and characterization of the gene, recent studies have contributed to our understanding of the structural and functional aspects of the glc-6-P transporter. Recently, a highly specific Glc-6-Pase inhibitor, S 5627, a synthetic analogue of chlorogenic acid, has been shown to interact at two sites on the transporter, one with high affinity and one with low affinity (5). Also, the involvement of two critical thiol groups in the T_1 transport activity has been demonstrated using the sulfhydryl reagent N-ethylmaleimide (28).

GLUCOKINASE

Hexokinases (HKs) catalyze the conversion of glucose to glc-6-P in the liver. In liver, two HK isoforms are expressed, HK I and HK IV or glucokinase (GK). Because GK is the major HK expressed and has a relatively high K_m for glucose, that is in the physiological range, it is thought to play a major role in glucose homeostasis in the liver (114). After feeding, when glucose is being taken up by the liver, GK metabolically traps glucose as glc-6-P for the liver cell to metabolize. However, when the liver is producing glucose, it is imperative that newly formed glucose from gluconeogenesis or glycogenolysis not become a substrate for GK. Phosphorylation of this glucose pool would lead to futile cycling and prevent net formation of glucose. Thus, GK must be regulated. This necessary down-regulation and/or inhibition of GK can be viewed in short- and long-term changes.

Acute Regulation of Glucokinase

GK was shown to be inhibited by the long-chain acyl-CoA compounds and N-acetyl-glucosamine, which would be elevated in times when glucose production by the liver would be needed (82, 101). The activity of GK can be inhibited by the GK regulatory protein (GKRP) (108–110). In the presence of high fructose-6-P, this protein binds GK and forms an inhibitory complex (105). This inhibition is relieved by fructose-1-P in a competitive manner. It is thought that in the fasted state, the level of fructose-6-P derived from glucose would result in the partial inhibition of GK by GKRP, thereby decreasing net liver glucose uptake and decreasing phosphorylation of newly formed glucose. By use of immunohistochemistry, GK has been localized to the nucleus of hepatocytes and is translocated from the nucleus to the cytoplasm in response to high glucose concentrations (103). Conversely, this sequestration of GK in the nucleus in the fasted or low glucose state would limit catalysis by GK and minimize glucose/glc-6-P futile cycling. This would allow movement of glucose out of the liver cell. Recently, the GKRP has been shown to interact with GK in the nucleus in hepatocytes under low glucose conditions (20). It is not clear how the GK-GKRP complex is anchored in the nucleus or whether the complex forms solely in the nucleus. These interactions, under low glucose conditions, support the model that glucose production in the liver requires sequestration of the catalytic activity of GK. In addition, not only is GK sequestered, it is bound to a protein that inhibits its catalytic activity (97).

Regulation of Hepatic Glucokinase Gene Expression

The GK gene is transcribed from two widely separated (35 kb) promoters, an upstream one that is β-cell specific and a downstream one that is hepatocyte specific (89). The regulation of the hepatocyte-specific promoter is complex and

less well understood than that of the β-cell–specific promoter. Insulin is known to increase hepatic GK transcription, whereas glucagon, acting via cAMP, decreases gene transcription (40, 41, 43, 52). This pattern of regulation is consistent with the metabolic profile where GK would be increased in the fed state (high insulin) during glucose utilization and suppressed in the fasted state (high glucagon) when glucose production would be required. Also, thyroid hormone and biotin increase GK gene transcription in liver (27, 66).

Iynedjian et al (42) recently reported that a 1-kb fragment of the downstream promoter in rats is transcriptionally active in primary hepatocytes, but it is not regulated by insulin. Generally, the *cis*-regulatory elements that determine hepatocyte-specific expression and regulation of the hepatic GK isoform are largely undefined because no isolated DNA fragment has been shown to confer both hepatocyte-specific and hormone-regulated expression to a reporter gene. In a transgenic mouse model, the GK locus spanning from -55 to $+28$ kb relative to the liver transcription start site contained all the regulatory and tissue-specific sequences required for the expression of both isoforms (67). It appears there is more progress to be made on the mapping of the liver GK regulatory sites.

GLUT 2

A requisite for glucose production from the liver is transport of glucose, formed from either gluconeogenesis or glycogenolysis, out of the liver. This transport function is carried out by GLUT 2. GLUT 2 is a member of a family of integral membrane protein glucose transporters characterized by 12 membrane-spanning domains, named GLUT 1–8. The structure, function, and regulation of major glucose transporters was reviewed by Olson & Pessin (81). GLUT 2 is expressed primarily in the liver but also in the intestine, kidney, and pancreatic β-cells (100). It is a low-affinity, high-capacity glucose transport protein that is distinguished from GLUT 1, GLUT 3, and GLUT 4, which have a much greater affinity for glucose. Using 3-O-methyl-D-glucose as a transport substrate, Craik & Elliott (30) demonstrated that transport is symmetrical, with no evidence of substrate stimulation from the *trans* side. Therefore, hepatic GLUT 2 is well suited to facilitate glucose uptake or efflux in the concentration range of glucose necessary to maintain glucose homeostasis, depending on the nutritional and endocrine states.

Structure/Function

Functional and structural differences between GLUT 2 and the high-affinity glucose transporters have been delineated from studies using genetically engineered chimeras of GLUT 2 with GLUTs 1, 3, and 4 (low affinity:high affinity

pairs). The substitution of GLUT 2 regions into the other isoforms revealed a change from the GLUTs 1, 3, and 4 characteristics to GLUT 2 characteristics with respect to kinetic properties and substrate specificity and thereby identified critical regions, and visa versa. Regions of GLUT that control differences in substrate specificity between the types of GLUTs have also been similarly defined by this methodology.

Using this approach with GLUT 2–GLUT 3 chimeras and 2-deoxyglucose (2-DOG) as a substrate, Arbuckle et al (2) showed that the seventh putative transmembrane-spanning helix is intimately involved in the selection of the substrate and determination of its K_m for transport. Also, a region between the end of helix 7 and the end of helix 10, together with sequences in the N-terminal half of the transporter, have been postulated to participate in substrate recognition and transport. Buchs et al (21), in a chimera study of GLUT 2 and GLUT 4 using expression in *Xenopus* oocytes, demonstrated that both the transmembrane domains 7–12 and the COOH-terminus of GLUT 2 are responsible for its distinctive low glucose affinity. Noel & Newgard (68) carried out a chimeric study of GLUT 2 and GLUT 1 using a recombinant adenovirus vector (see 1) in the kidney cell line, CV-1. Constructs made up of mostly GLUT 1 with the C-terminal tail of GLUT 2 had a significantly lower K_m for 2-DOG substrate but showed no change in ability to use the alternate substrates fructose, arabinose, and streptozotocin, which are transported by GLUT 2 but not GLUT 1. In the converse chimeric construct, no change in K_m for 2-DOG was observed, but a decreased capacity for the alternate substrates was. An Asp-62 to Gln substitution prevented attachment of an N-linked oligosaccharide and led to a 2.5-fold increase in the K_m for 2-DOG; it had no effect on alternate substrates relative to native GLUT 2. Taken together, these observations not only demonstrate the importance of the C-terminal region, they also illustrate that the specificity of transport is independent of affinity.

Short- (Translocation) and Long-Term (Transcription) Regulation

There is no evidence that GLUT 2 follows anything other than a direct path to the plasma membrane after synthesis or that it participates in regulated translocation, as is the case with the insulin-sensitive transporter, GLUT 4. In some ways the regulation of GLUT 2 parallels Glc-6-Pase gene regulation (see above).

GLUT 2 expression has been reported to be up-regulated by glucose, like the expression of PK, fatty acid synthase, and insulin (90). The up-regulation of PK and fatty acid synthase requires metabolism to glc-6-P and is potentiated by insulin, whereas GLUT 2 up-regulation is counteracted by insulin. One can speculate that because metabolism of glucose is required, the regulation of GLUT 2 expression may be related to the levels of the pentose phosphate

intermediate xyulose-5-phosphate, which is thought to play a role in the regulation of carbohydrate metabolism genes like Glc-6-Pase and PK (3, 54).

Antoine et al (1a) demonstrated a requirement for GLUT 2 in the response of the L-type PK to glucose in liver-derived cells. GLUT 2 expression is necessary to allow glc-6-P depletion via glucose export. Antoine el al (1a) propose that in the absence of GLUT 2, glucose would recycle to glc-6-P, which would lead to the synthesis of the downstream metabolite that stimulates PK expression. Hence, regulation would be lost and PK expression would be constitutively high. This hypothesis, however, is put forth without direct measurement of Glc-6-Pase gene expression or activity.

In diabetic rats after sequential transient hypoglycemia followed by hyperglycemia (48 h), the expression of GLUT 2 is up-regulated, as evidenced by two- to threefold increases in mRNA and protein levels (22). This pattern of gene regulation follows that seen with the gluconeogenic enzyme, PEPCK. Additionally, GLUT 2 is more highly expressed in the periportal cells of the liver, also a characteristic of the gluconeogenic enzymes (100). This type of regulation would support the argument that up-regulation of GLUT 2 was more critical in glucose export from the liver to maintain glucose homeostasis than in import, and during times when the liver is importing glucose, basal levels of GLUT 2 expression are sufficient to support oxidation. However, evidence for a pathway of glucose release alternative to GLUT 2 has recently appeared (36a).

Glucose transport out of the lumen of the ER is also required to have net hepatic glucose output. This is because of the location of the catalytic site of Glc-6-Pase on the luminal surface of the ER. Historically, this glucose transporter has been treated as a component of the Glc-6-Pase system (79, 99); it is thought to be of the same high-capacity, low-affinity type of glucose transporter as is seen in the plasma membrane of the liver (i.e. GLUT 2) based on similar requirements. Reports of a distinct ER glucose transporter have not been substantiated (24, 112).

HYPOTHESIZED CONTRIBUTIONS OF GLUCOKINASE AND MULTIPLE ACTIVITIES OF GLUCOSE-6-PHOSPHATASE TO THE ESTABLISHMENT AND MODIFICATION OF BLOOD GLUCOSE LEVELS

As an introduction to this section, the reader is directed to references 70, 72, and 73, where the kinetic and physiologic concepts underlying what we refer to as the "tuning/retuning hypothesis," were first described. A comprehensive, updated description is given in two recent reviews (33, 79). In these earlier

descriptions of the hypothesis, the term "plateau blood glucose level" was used in reference to the steady-state blood glucose concentrations achieved in the post-absorptive state for a period of several hours beginning in humans approximately three to four hours after glucose ingestion.

The "tuning" of the liver refers to the establishment of the functional levels of the hepatic enzymes glucokinase, glc-6-P phosphohydrolase, and biosynthetic activities of Glc-6-Pase which poise the liver to play a central role (along with other physiologic factors) in the maintenance of blood glucose concentrations at normal, ambient values. "Retuning" is used to refer to hormonally or otherwise induced changes in functional levels of these enzymes to repoise or reposition the liver for its still-central role in blood glucose homeostasis, but at an altered post-absorptive glucose concentration as, e.g. in the hyperglycemia of diabetes, or other conditions described below.

The liver's critical contribution to "tuning" or establishing steady-state blood glucose concentration through a balance between the apposing actions of the hepatic enzymes of glucose phosphorylation and glc-6-P hydrolysis is made clear in Figure 22 of Reference 70, Figures 6 and 12A of Ref. 72, and Figures 2b of Ref. 73. Changes in levels of these enzyme activities in response to hormonal or other perturbations are proposed to contribute importantly to altered steady-state post-absorptive concentrations of blood glucose (see fig. 22C in ref. 70, fig. 12B in ref. 72, and fig. 2C in ref. 73).

Critical to the hypothesis, with the Glc-6-Pase system where K_m values for glc-6-P and carbamyl-P are much greater than their physiologic concentrations in liver, activities with glc-6-P and carbamyl-P behave kinetically independently—i.e. as though totally independent enzymes were involved with the two substrates (80a).

Between 1974 and 1976, Nordlie and associates (70–72) first proposed that biosynthetic activity of Glc-6-Pase (Reactions 3 and 4) plays a role, along with its hydrolytic activity and GK, in hepatic glucose homeostasis. At the time, this hypothesis was based on kinetic considerations, the opposite responses of GK and Glc-6-Pase phosphotransferase to insulin deprivation, large differences in the maximal activities of Glc-6-Pase phosphotransferase and GK, and demonstrated metabolic channeling involving carbamyl-P synthesis by mitochondrial carbamyl-P synthase I and subsequent glucose phosphorylation via carbamyl-P:glucose phosphotransferase activity of the Glc-6-Pase system (Reaction 3).

Simply stated (70, 73, 79), the tuning/retuning hypothesis (as the author termed it) depends on the differences in K_m, glc (Michaelis constant for glucose) for GK (approximately 10 mM) and phosphotransferase activity of Glc-6-Pase (approximately 40 mM at low carbamyl-P concentrations). It follows from kinetic analysis that an increase in the ratio of phosphotransferase activity of Glc-6-Pase/GK will contribute to an increase in the post-absorptive steady state blood glucose level. Because GK requires insulin as an inducer and Glc-6-Pase

increases in diabetes, the ambient level of blood glucose which the liver is metabolically adjusted to maintain increases progressively with the severity of diabetes. The hypothesis is applicable to rationalize variations in blood glucose levels in experimental diabetes, NIDDM, maturity-onset diabetes in the young, von Gierke patients, hyperglycemia of aging, and marked, normal variations in blood glucose among various species (e.g. birds, frogs, humans) (79).

Inherent in the hypothesized tuning/retuning mechanism are several metabolic regulatory advantages (79). It provides for great sensitivity to even small variations in blood glucose levels over a broad range. Rather than being an either/or, on/off mechanism, it provides for a gradation of response of blood glucose concentration. Extent of response of ambient blood glucose concentration is correlated closely with the degree of insulin insufficiency or insulin insensitivity. The hypothesis is also applicable to other conditions of varying glycemia, independent of the diabetic state.

Critical to this hypothesis is the function of the phosphotransferase activity of Glc-6-Pase in hepatic glucose phosphorylation under physiologic or near-physiologic conditions. Several recent "paradoxical" reports of studies involving regulation at the Glc-6-Pase gene level provide supportive evidence. Both an elevation in exogenous glucose concentration (3, 4, 53, 54) and increased fru-2,6-P_2 (3) correlated with an increase in expression of the Glc-6-Pase catalytic unit gene. Because the hydrolytic activity of Glc-6-Pase produces glucose and because fru-2,6-P_2 is a stimulator of glycolysis (at the PFK level) rather than of gluconeogenesis, these observations may be considered paradoxical (3, 85, 88). But the situation is not so paradoxical if one accepts that phosphorylation of glucose, at comparatively high blood glucose levels, via its biosynthetic function is also a physiologically important role for Glc-6-Pase.

Another recent study at the gene-regulation level indicates that overexpression of Glc-6-Pase catalytic unit gene in hepatocytes treated with AdCMV-Glc-6-Pase, again "paradoxically," leads to hyperglycemia, as in NIDDM when Glc-6-Pase also increases (95, 102). Again, interpreted in terms of the tuning/retuning hypothesis, the observed hyperglycemia is predictable based on the increase in the ratio of phosphotransferase activity of Glc-6-Pase system (which increases as the Glc-6-Pase catalytic unit is elevated)/GK (which is unchanged in this experimental model). In this situation, the alteration in this ratio, with resultant increase in post-absorptive steady-state blood glucose level, is achieved independently of insulin involvement.

A number of other pieces of experimental evidence indicative of hepatic glucose phosphorylation by a high-K_m, glc enzyme other than GK and supportive of a role for the biosynthetic activity of the Glc-6-Pase system in hepatic glucose metabolism also have emerged during the past five years. Several of these studies have been reviewed in detail already (33); a few of the most recent are deserving of brief mention here as well. Wals & Katz (113) have provided further

experimental evidence of phosphorylation of glucose in liver by an enzyme or enzymes with a K_m, glc greater than that of GK. Henly et al (38) demonstrated vigorous rates of cycling between glc-6-P and glucose in hepatocytes from both normal and streptozotocin-diabetic rats using high levels of glucose; they also showed a glucose phosphorylation rate only 30% below the normal control rate, even though GK is largely lost in diabetes. This is consistent with the observation (74) of net glucose uptake in the virtual absence of GK in perfused livers of diabetic rats at and above 3 mM glucose when 3-mercaptopicolinate, a potent inhibitor of glc-6-P phosphohydrolase but not phosphotransferase activity of the Glc-6-Pase system (17), is present to inhibit rehydrolysis of Glc-6-P.

An inverse relationship between ureagenesis and glycogenesis in isolated perfused livers has been shown to revolve about the availability of carbamyl-P to the two processes (18). These studies involved the use of the specific inhibitor norvaline, which targets the first committed step in ureagenesis, ornithine transcarbamylase. When ureagenesis is shut down, glycogenesis from glucose concomitantly rises. The authors (18) proposed that this is because carbamyl-P is then diverted to glucose phosphorylation via biosynthetic activity of the Glc-6-Pase system. Further, ethoxyzolamide, an inhibitor of mitochondrial carbonic anhydrase, lowers carbamyl-P formation and concentration as a consequence of decreased HCO_3 availability. Both ureagenesis and glycogenesis from glucose were then reduced as a result of diminished carbamyl-P (18). Other studies, in which glutamine and proline were compared as substrates for ureagenesis and glyconeogenesis, also support the contention that carbamyl-P may serve as a phosphoryl donor for glucose phosphorylation in liver, preliminary to glycogen synthesis (19). Metabolism of a single molecule of glutamine provides, concomitantly, both for ammonium ion for carbamyl-P synthesis and for aspartate formation. Both carbamyl-P and aspartate are necessary substrates for ureagenesis. In contrast, a proline molecule, when metabolized to ammonium ion and then carbamyl-P, does not provide aspartate for ureagenesis; the carbamyl-P generated may then be available for other hepatic cellular processes. Studies just published (83) indicate a direct correlation between glycogen synthase (55) and biosynthetic activity of Glc-6-Pase in terms of their responses to reversal of chloride ion inhibition.

FUTURE DIRECTIONS OF STUDIES OF SOME ABERRANT STATES

In 1929, von Gierke (111) reported on a patient with glycogenosis of the liver and kidneys. This was to become classified as glycogen storage disease type I (GSD type 1) and some twenty years later was attributed to a deficiency in the enzyme microsomal Glc-6-Pase (29). Forty-five years after this attribution, mutations in this enzyme that lead to GSD type 1 were identified as a result of

the isolation and characterization of cDNAs coding for the human liver Glc-6-Pase gene (48). In 1975, a multicomponent model for Glc-6-Pase involving a glc-6-P translocase was proposed (8), and later a subtype of GSD type 1, called type 1b, was attributed to the absence of putative glc-6-P translocase (46, 65). Now, 22 years after the initial proposal, a putative glc-6-P translocase has been identified and isolated, and patients with GSD type 1b have been shown to have mutations in this locus (36). Mutations in the Glc-6-Pase system have a profound impact on the ability of the liver to export glucose, where it can only come from limited hydrolysis of glycogen to form glucose. In the absence of hydrolytic activity or transport components, glc-6-P is diverted to glycogen, leading to the hepatomegaly seen in GSD type 1. Frequent feeding regimens are necessary to give patients with GSD type 1 relief from diversion of glc-6-P into glycogen and the inability of their livers to produce glucose. With the above mentioned recent advances in the molecular biology of the Glc-6-Pase system, we are on the threshold of realizing genetic therapies for treatment of GSD.

The enhanced production of glucose by the liver is a major contributor to hyperglycemia, the hallmark of diabetes. The proteins discussed in this review that are involved in this process—GK, GLUT 2, and Glc-6-Pase—have all been considered as candidate genes in the etiology of diabetes. As we have tried to illustrate in this review, they are likely involved because of how their actions affect the production of glucose by the liver.

GK has been shown to be a diabetes gene where a number of mutations in the gene lead to the type 2 maturity onset diabetes in the young phenotype (14). Kinetically compromised (i.e. with a decreased V_{max} and/or increased K_m for glucose or ATP) or inactive enzyme leads to the inability of the liver to trap glucose as glc-6-P. A GK translocation defect preventing the glucose-dependent recruitment of GK from the nucleus to the cytosol would decrease glucose disposal and raise blood glucose levels (20).

If Glc-6-Pase is involved in the etiology of diabetes, this could be through the regulation of its gene expression. This could occur either directly, by mutation in hormone response element sequences, or indirectly, by mutations in transcription factor DNA binding sequences that interact with the Glc-6-Pase gene and increase its expression (up-regulation). This was shown by the establishment of a prediabetic state by overexpression of the catalytic subunit of Glc-6-Pase via adenovirus vectors (102). This study also showed that changes in Glc-6-Pase alone could not lead to the diabetic state (102). However, as pointed out above, increases in the activities of this enzyme concomitant with a loss of GK can effectively retune the liver for its important contribution to the maintenance of blood glucose at the hyperglycemic, "diabetic" level. Although a genetic component has not been established, any cause that would lead to higher-than-normal levels of Glc-6-Pase would contribute metabolically to excess glucose production by the liver, thereby establishing hyperglycemia.

SUMMARY

Glucose is a vital cellular fuel which must be tightly regulated to maintain blood glucose homeostasis. Levels of blood glucose that are too high or too low are detrimental, causing diabetes or hypoglycemia. When glucose is not being ingested, it is the role of the liver to produce this nutrient, either from its glucose stores (glycogenolysis) or de novo, from three carbon precursors (gluconeogenesis).

We reviewed the regulation of proteins involved in the process of hepatic glucose production. We focused on the terminal steps: (*a*) hydrolysis of glc-6-P via Glc-6-Pase, (*b*) its antithetical counterpart (glucose/glc-6-P substrate cycle), glucokinase, and (*c*) transport of the final product, glucose, to the blood. Necessarily, the regulation of hepatic glucose output is complex, involving both acute and long-term effects. Examination of this regulation has revealed regulatory paradoxes (e.g. up-regulation of Glc-6-Pase by glucose and Fru-2,6-P_2) that expose inconsistencies in our understanding of liver metabolism. Therefore, we need to keep reexamining basic principles of carbohydrate metabolism and to consider mechanisms like the glc-6-P synthetic activity of Glc-6-Pase, which may explain observed paradoxes.

Also, explosive advances using molecular biology approaches have led to a better understanding of the regulation of hepatic glucose production. They have brought us closer to understanding long-standing problems, e.g. the mechanism by which glucose formed in the lumen of the ER traverses the cytosol for delivery through the plasma membrane to the bloodstream. Although the translocation of GK to the nucleus to form an inactive conformation in conditions of low glucose influx contributes to the likely mechanism, still a tightly coupled architecture of multiprotein complexes may be necessary and has long been postulated (70, 72) to channel glucose to the bloodstream. Structural studies, derived from the results of molecular biology approaches, are now needed to unravel the subtle complex architecture of the process of hepatic glucose production.

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