Carbon Flux via the Pentose Phosphate Pathway Regulates the Hepatic Expression of the Glucose-6-phosphatase and Phosphoenolpyruvate Carboxykinase Genes in Conscious Rats*

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Hepatic gene expression of P-enolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (Glc-6-Pase) is regulated in response to changes in the availability of substrates, in particular glucose (Glc; Massillon, D.; Barzilai, N.; Chen, W.; Hu, M., and Rossetti, L. (1996) J. Biol. Chem. 271, 9871–9874). We investigated the mechanism(s) in conscious rats. Hyperglycemia per se caused a rapid and marked increase in Glc-6-Pase mRNA abundance and protein levels. By contrast, hyperglycemia decreased the abundance of PEPCK mRNA. Importantly, inhibition of glucokinase activity by glucosamine infusion blunted both the stimulation of Glc-6-Pase and the inhibition of PEPCK gene expression by Glc, suggesting that an intrahepatic signal (metabolite) generated by the metabolism of glucose at or beyond Glc-6-P was responsible for the regulatory effect of Glc.

The effect of Glc on the L-type pyruvate kinase gene is mediated by xylulose-5-P (Doiron, B.; Cuif, M.; Chen, R., and Kahn, A. (1996) J. Biol. Chem. 271, 5321–5324). Thus, we next investigated whether an isolated increase in the hepatic concentration of this metabolite can also reproduce the effects of Glc on Glc-6-Pase and PEPCK gene expression in vivo. Xylitol, which is directly converted to xylulose-5-P in the liver, was infused to raise the hepatic concentration of xylulose-5-P by ~3-fold. Xylitol infusion did not alter the levels of Glc-6-P and of fructose-2,6-biphosphate. However, it replicated the effects of hyperglycemia on Glc-6-Pase and PEPCK gene expression and resulted in a 75% increase in the in vivo flux through Glc-6-Pase (total glucose output).

Glucose-6-phosphatase (Glc-6-Pase) catalyzes the phosphohydrolysis of glucose-6-phosphate to glucose and is the final step for the release of glucose by the liver (1). The catalytic unit is localized in the endoplasmic reticulum where it is anchored by a stabilizing protein (1–4). It has long been recognized that hepatic Glc-6-Pase activity is markedly regulated by changes in hormones (insulin, dexamethasone, and cAMP) and nutritional status (1, 2, 5–11). The recent cloning of the catalytic portion of Glc-6-Pase (8, 12–15) has shed light into the gene regulation of this hepatic enzyme. Its hepatic mRNA levels are increased in insulin-deficient diabetes and starvation, and decreased by refeeding and insulin treatment (2, 16–21).

We have recently demonstrated that the marked increase in Glc-6-Pase mRNA and protein levels in the liver of diabetic rats was restored to normal levels following normalization of the plasma glucose concentrations by either insulin or the glycosuric agent phlorizin (19). Since phlorizin administration failed to normalize Glc-6-Pase gene expression when hyperglycemia was maintained by glucose infusion (19), we suggested that Glc-6-Pase gene expression in the diabetic liver is regulated by glucose independent of insulin. This preliminary observation has received support by a recent comprehensive study in primary cultured hepatocytes which demonstrated stimulation of Glc-6-Pase gene expression by glucose and fructose 2,6-biphosphate (22). Thus, Glc-6-Pase belongs to a family of genes whose expression is regulated by carbohydrates. In particular, this family includes genes coding for key enzymes within the gluconeogenic, glycolytic, and lipogenic pathways, such as L-type pyruvate kinase (23, 24), PEPCK (25, 26), and fatty acid synthase (27, 28).

While changes in the plasma glucose concentration are likely to regulate the gene expression of liver enzymes in the intact organism as well (19), the impact of these changes on in vivo glucose fluxes has not been delineated. It has long been recognized that acute hyperglycemia decreases HGP in perfused liver (29) and in non-diabetic animals (10, 30, 31) and humans (32). This decrease is associated with increased flux through glucokinase (increased glucose cycling), with no measurable changes in the in vivo fluxes through gluconeogenesis and Glc-6-Pase (10). Thus, in the presence of hyperglycemia, it may be difficult to isolate the impact of the stimulation of hepatic Glc-6-Pase gene expression on hepatic glucose fluxes.

The aim of the present work was to test the hypothesis that the flux of glucose through glucokinase modulates Glc-6-Pase gene expression in conscious rats. We also wished to determine whether glucose itself or an intracellular metabolite generated by glucose metabolism is responsible for this stimulatory effect on the expression of the Glc-6-Pase gene. Our results indicate that, similar to what has been recently observed for the hepatic pyruvate kinase gene in cultured hepatocytes (33), an intrahepatic signal generated by the metabolism of glucose in the nonoxidative branch of the pentose phosphate pathway (Fig. 1A) can reproduce the stimulatory effect of glucose on the expression the Glc-6-Pase gene in vivo. Most important, this
stimulation resulted in a marked increase in the rate of glucose production. We propose that the nonoxidative branch of the pentose phosphate shunt represents a glucose sensing regulatory pathway which activates a feedback control system limiting the size of the Glc-6-P pool in response to sustained increases in the rate of hepatic glucose phosphorylation.

MATERIALS AND METHODS

Animals—Male Sprague-Dawley rats (Charles River Breeding Laboratories, Inc., Wilmington, MA) were used in all studies. Rats were housed in individual cages and subjected to a standard light (6 a.m. to 6 p.m.) and dark (6 p.m. to 6 a.m.) cycle. Five to seven days before the in vivo study, rats were anesthetized with an intraperitoneal injection of pentobarbital (50 mg/kg body weight) and indwelling catheters were inserted into the right internal jugular vein and in the left carotid artery. The venous catheter was extended to the level of the right atrium and the arterial catheter was advanced to the level of the aortic arch (34, 35).

Insulin/Somatostatin/Glucose/Xylitol Infusions—Studies were performed in awake, unstressed, chronically catheterized rats using a combination of the pancreatic and hyperglycemic clamp techniques (10, 36, 37) (Fig. 1B). All rats were fasted for 6 h before the in vivo studies. At the beginning of the basal period and 120 min before starting the glucose infusion, prior to the somatostatin/xylitol infusions, a primed continuous infusion of regular insulin (1.5 milliunits/kg min) was initiated and maintained throughout the remainder of the study. Briefly, in protocol 1, a primed continuous infusion of somatostatin (1.5 μg/kg min) and regular insulin (−0.7 milliunits/kg min) were administered, and a variable infusion of a 25% glucose solution was started at time 0 and periodically adjusted to clamp the plasma glucose concentration at −7 mmol/l for 5 h (euglycemic studies) or at −7 mmol/l for 2 h followed by 1, 3, or 5 h at −17 mmol/l (hyperglycemic studies). When indicated, glucosamine (GlcN) was infused at a rate of 30 μmol/kg min, designed to elevate and maintain the plasma GlcN concentrations at −2 mmol/l. During the first 2 h of the studies (euglycemic period) the rate of insulin infusion was adjusted as required to maintain normoglycemia during the somatostatin infusion. The average insulin infusion required to maintain normoglycemia in all groups was 0.7 ± 0.1 milliunits/kg min, without the need for glucose infusion. In one group of rats the rate of insulin infusion was increased to 18 milliunits/kg min for 3 h while the plasma glucose concentration was raised and maintained at −17 mmol/l by a variable infusion of glucose. In protocol 2, a primed continuous infusion of somatostatin (1.5 μg/kg min) and regular insulin (1.0 milliunits/kg min) were administered, and a variable infusion of a 25% glucose solution was started at time 0 and periodically adjusted to clamp the plasma glucose concentration at −7 mmol/l for 5 h. During the last 3 h of the studies, vehicle or xylitol (30 μmol/kg min) were also infused. Blood samples (−25 μl) were taken every 10 min to monitor the plasma glucose concentrations and adjust the rates of glucose infusion. Plasma samples for determination of plasma insulin, glucagon, and FFAs were obtained every 30 min during the study. At the end of the insulin infusion, rats were anesthetized (pentobarbital 60 mg/kg body weight, intravenously), the abdomen was quickly opened, portal vein blood obtained and liver and pancreas were freeze-clamped in situ with aluminum tongs precooled in liquid nitrogen. The time from the injection of the anesthetic until freeze-clamping of the liver was less than 45 s. Tissue samples were stored at −80 °C for analysis. The protocol was approved by the Institutional Animal Care and Use Committees of Albert Einstein College of Medicine.

Immunoblotting Analysis—Microsomes were prepared according to van de Verve et al. (11, 19, 36). Briefly, liver tissue (100 mg) was homogenized in 10 volumes of a Tris sucrose/phenylmethylsulfonyl fluoride buffer (50 mm Tris buffer, pH 7.3, 250 mm sucrose, 1 mm phenylmethylsulfonyl fluoride, and 1 mm EGTA). This homogenate was centrifuged for 10 min at 10,000 × g; the cytosol was then centrifuged for 1 h at 100,000 × g, and the pellet resuspended in 1 ml of Tris sucrose/phenylmethylsulfonyl fluoride buffer. The resuspended pellet was incubated at 4 °C for 30 min in the presence of Triton X-100 at a final concentration of 0.1%. Protein content was measured by the Bio-Rad protein assay (Bio-Rad). The isolated RNA was assessed for purity by the ratio of absorbance at 260 and 280 nm. Total RNA was isolated from freeze-clamped liver tissues according to the Trizol method (Life Technologies, Gaithersburg, MD). The isolated RNA was assessed for purity by the ratio of absorbance at 260 and 280 nm. The RNA was visualized with ethidium bromide and transferred to a Hybond-N+ membrane (Amersham). We used a 1.25-kilobase Eco-HindIII Glc-6-Pase cDNA, a 2.2-kilobase glucokinase cDNA (kindly provided by Dr. Mark Magnuson, Vanderbilt University), and a PEPCK cDNA (kindly provided by Dr. Richard Hanson, Case Western Reserve University) which were labeled with [α-32P]dCTP, using the Random Prime Labeling system (Life Technologies). Prehybridization was per-
formed for 4 h at 42 °C in 5 × SSC, 50% formamide (v/v), 5 × Denhardt’s, 100 µg/ml salmon sperm DNA, 1% SDS, 100 mM phosphate buffer, pH 6.5, 10 mM EDTA. Hybridization was carried out for 16 h in the same buffer with the 32P-labeled probe. The filters were washed 3 times for 10 min in 2 × SSC, 0.1% SDS at room temperature and 2 times in 0.1 × SSC, 0.1% SDS for 30 min at 55 °C, then exposed to Fuji x-ray films for 12–48 h at ~80 °C with intensifying screens. Quantification was done by scanning densitometry.

**Analytical Procedures—** Plasma glucose was measured by the glucose oxidase method (Glucose Analyzer II, Beckman Instruments, Inc., Palo Alto, CA). Plasma insulin was measured by radioimmunoassay, using rat and porcine insulin standards. The plasma concentration of FFA was determined by an enzymatic method with an automated kit according to the manufacturer’s specifications (Waco Pure Chemical Industries, Osaka, Japan). Plasma [3H]glucose radioactivity was measured in duplicates in the supernatants of Ba(OH)2 and ZnSO4 precipitates (Somogyi procedure) of plasma samples (20 µl) after evaporation to dryness to eliminate tritiated water. Fructose 2,6-biphosphatase was extracted at 80 °C in 0.1 M NaOH and measured using the 6-phosphofructo-1-kinase assay (38). Xylose-5-P concentration was assayed according to the method of Casazza and Veech (39, 40). Uridine diphosphoglucose and uridine diphosphogalactose concentrations and specific activities in the liver were obtained through two sequential chromatographic separations, as previously reported (10). Plasma glucosamine concentrations were determined by high performance liquid chromatography following quantitative derivatization with phenyl isothiocyanate as described previously (41). Differences between groups were determined by ANOVA analysis of variance. All values are presented as the mean ± S.E.

Terminology (10)—The term total glucose output (TGO) is intended as total in vivo flux through Glc-6-Pase. The term hepatic glucose production (HGP) is intended as the net rates of Glc-6-P dephosphorylation to glucose. Finally, glucose cycling is defined as the input of extracellular glucose into the Glc-6-P pool followed by exit of plasma glucose directly or by a metabolite derived from glucose metabolism.

**Calculations—** Under steady-state conditions for plasma glucose concentrations, the rate of glucose disappearance equals the rate of glucose production (HGP) during the euglycemic and hyperglycemic clamp studies. In the presence of similar plasma insulin and glucagon levels, 2–3 h hyperglycemia per se inhibited HGP by 40% compared with either basal studies (saline infusion) or time control studies at euglycemia. However, this suppression of HGP could not be detected following 4–5 h of hyperglycemia. In fact, HGP was similar between 4 and 5 h of hyperglycemia and euglycemia. While TGO was not significantly affected by hyperglycemia at 3 h, it was significantly increased (by 58%) following 5 h at hyperglycemia versus euglycemia. As previously reported (10), hyperglycemia markedly stimulated the rate of glucose cycling. However, this rate was further increased between 3 and 5 h of hyperglycemia, probably reflecting the marked stimulation of the flux through Glc-6-Pase.

**Effect of Glucose per se on Hepatic Glucose Fluxes in Conscious Rat—** Fig. 2A depicts the rate of hepatic glucose production (HGP) during the euglycemic and hyperglycemic clamp studies. In the presence of similar plasma insulin and glucagon levels, 2–3 h hyperglycemia per se inhibited HGP by 40% compared with either basal studies (saline infusion) or time control studies at euglycemia. However, this suppression of HGP could not be detected following 4–5 h of hyperglycemia. In fact, HGP was similar between 4 and 5 h of hyperglycemia and euglycemia. While TGO was not significantly affected by hyperglycemia at 3 h, it was significantly increased (by 58%) following 5 h at hyperglycemia versus euglycemia. As previously reported (10), hyperglycemia markedly stimulated the rate of glucose cycling. However, this rate was further increased between 3 and 5 h of hyperglycemia, probably reflecting the marked stimulation of the flux through Glc-6-Pase.

**Effect of Glucose per se on Hepatic Glc-6-Pase mRNA Levels in Conscious Rat—** To examine in vivo the effect of hyperglycemia per se on the gene expression of key hepatic enzymes, we infused somatostatin to inhibit the endogenous secretion of insulin and glucagon, replaced insulin either at basal or high levels, and infused glucose at a variable rate to achieve and maintain either euglycemia or hyperglycemia for 1, 3, or 5 h (Fig. 1B). The relative abundance of Glc-6-Pase mRNA was examined by Northern blot analysis using a 1.25-kilobase cDNA that recognizes the catalytic portion of this enzyme. Fig. 3A shows that hyperglycemia markedly increased the Glc-6-Pase mRNA in the liver of nondiabetic rats. Analysis of the time course of this effect demonstrates a rapid increase in the mRNA abundance within 1 h reaching a maximal value (more than 5-fold increase) at 3 and 5 h.

**Role of Glucose Phosphorylation—** The stimulatory effect of glucose on Glc-6-Pase gene expression could be mediated by glucose directly or by a metabolite derived from glucose metabolism. The first step in hepatic glucose metabolism is its phosphorylation by glucokinase. Glucosamine is a potent competitive inhibitor of glucokinase and it has been previously used for this purpose in vitro (42) and in vivo (37, 43). Specifically, we

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**Table I**

| Group          | n | Glucose | FFA | Insulin | Glucagon |
|----------------|---|---------|-----|---------|----------|
| EUGLYCEMIA     | 7 | 7.2 ± 0.2 | 0.52 ± 0.09 | 31 ± 5 | 158 ± 11 |
| HYPERGLYCEMIA  | 8 | 17.6 ± 1.3* | 0.46 ± 0.12 | 40 ± 6 | 175 ± 18 |
| HYPERGLYCEMIA + GLUCOSAMINE | 6 | 17.5 ± 0.5* | 0.44 ± 0.06 | 39 ± 4 | 168 ± 16 |
| HYPERGLYCEMIA + INSULIN     | 5 | 17.1 ± 0.7* | 0.31 ± 0.19* | 489 ± 27 | 158 ± 19 |

* p < 0.01 versus euglycemia (1).

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**Table II**

| Group          | n | Glucose | FFA | Insulin | Glucagon |
|----------------|---|---------|-----|---------|----------|
| XYLITOL−       | 6 | 7.1 ± 0.4 | 0.51 ± 0.11 | 41 ± 4 | 164 ± 10 |
| XYLITOL+       | 5 | 7.6 ± 0.5 | 0.59 ± 0.14 | 42 ± 3 | 155 ± 14 |

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**Substrate Regulation of the Hepatic Glucose-6-phosphatase Gene**

Plasma samples for the determination of plasma FFA, insulin, and glucagon concentrations were sampled at 30-min intervals during the last 2 h of the in vivo studies. Plasma samples for the determination of plasma glucose concentrations were sampled at 10-min intervals. Values displayed in this table represent the average levels (± S.E.) during this time period.

| Group          | n | Glucose | FFA | Insulin | Glucagon |
|----------------|---|---------|-----|---------|----------|
| EUGLYCEMIA     | 7 | 7.2 ± 0.2 | 0.52 ± 0.09 | 31 ± 5 | 158 ± 11 |
| HYPERGLYCEMIA  | 8 | 17.6 ± 1.3* | 0.46 ± 0.12 | 40 ± 6 | 175 ± 18 |
| HYPERGLYCEMIA + GLUCOSAMINE | 6 | 17.5 ± 0.5* | 0.44 ± 0.06 | 39 ± 4 | 168 ± 16 |
| HYPERGLYCEMIA + INSULIN     | 5 | 17.1 ± 0.7* | 0.31 ± 0.19* | 489 ± 27 | 158 ± 19 |

* p < 0.01 versus euglycemia (1).
have recently shown that glucosamine, at plasma concentrations similar to those achieved in the present studies (37), decreases hepatic glucokinase activity in vitro (by 60%) and the flux through glucokinase in vivo (37). Thus, to delineate whether glucose needs to be phosphorylated to regulate the hepatic gene expression of Glc-6-Pase, we performed additional 3-h hyperglycemic clamp studies while the hepatic activity of glucokinase was markedly inhibited by maintaining throughout the plasma glucosamine concentration at ~2 mM. This duration of hyperglycemia (3 h) was selected since substantial accumulation of Glc-6-Pase mRNA was consistently observed at this time. The stimulatory effect of glucose on Glc-6-Pase gene expression (Fig. 3A) was prevented by the glucosamine-induced inhibition of glucokinase activity, indicating that glucose needs to be phosphorylated to exert its effect on the Glc-6-Pase gene.

Effect of Glucose per se on Hepatic PEPCK and Glucokinase (GK) mRNAs in Conscious Rats—To assess the specific effect of glucose on Glc-6-Pase enzyme expression, we next examined the effect of hyperglycemia per se on PEPCK and GK mRNA levels. Indeed, it has been previously shown that glucose independent of insulin decreases PEPCK gene transcription and accelerates the degradation of the PEPCK mRNA in streptozotocin-diabetic rats (26). Thus, liver PEPCK mRNA was used as a negative marker for Glc-6-Pase enzyme expression since its expression is expected to be regulated in an opposite fashion by glucose per se. In fact, PEPCK mRNA displayed opposite regulation to Glc-6-Pase mRNA. Hyperglycemia markedly, rapidly, and progressively decreased PEPCK mRNA starting within 1 h of hyperglycemia (Fig. 3B). Although the inhibitory effect of hyperglycemia was less pronounced on GK mRNA than on PEPCK mRNA, Fig. 4 shows that high glucose levels were also associated with a moderate decrease in the abundance of glucokinase mRNA both at the end of 3- and 5-h hyperglycemic clamp studies. Most important, glucosamine infusion completely blocked the effect of 3-h hyperglycemia on PEPCK and GK mRNA levels suggesting that, as for Glc-6-Pase mRNA, this effect required glucose phosphorylation (Figs. 3B and 4).

Effect of Glucose per se on Hepatic Glc-6-Pase Protein in Conscious Rats—To study if the increase in the mRNA coding for the catalytic portion of Glc-6-Pase was paralleled by a concomitant increase in the protein levels in liver microsomes, we performed Western blot analysis using a polyclonal antibody against the catalytic portion of Glc-6-Pase (16, 19, 36). As can be seen in Fig. 5, a marked (~3-fold) increase in the catalytic portion of Glc-6-Pase protein was detected within 3 h of hyperglycemia and sustained at 5 h.

Effect of Combined Hyperglycemia and Hyperinsulinemia on Glc-6-Pase mRNA—Under most physiologic conditions, increases in the plasma glucose concentration are tightly coupled with elevations in the circulating insulin levels. Thus, we next wished to examine whether hyperinsulinemia would effectively antagonize the induction of Glc-6-Pase gene expression by hyperglycemia. As can be seen in Fig. 6, hyperinsulinemia completely prevented the glucose-induced increase in liver Glc-6-Pase mRNA. In fact, in the presence of combined hyperglycemia and hyperinsulinemia, Glc-6-Pase mRNA abundance was similar to that observed in 6-h fasted euglycemic animals at basal insulin. Since the transcriptional regulation of the α-type pyruvate kinase gene by glucose has been recently shown to be mediated by xylulose-5-P (33), we hypothesized that an isolated increase in the hepatic concentration of this metabolite may reproduce the complex effects of Glc on Glc-6-Pase and PEPCK gene expression.

Effect of Xylitol Infusion on Hepatic Glucose Fluxes in Conscious Rat—The infusion of xylitol for 3 h resulted in a ~3-fold increase in the hepatic concentration of xylulose-5-P (from 19 ± 5 to 59 ± 11 nmol/g; p < 0.01). Conversely, the hepatic levels of Glc-6-P (289 ± 28 and 279 ± 36 nmol/g in xylitol+ and xylitol−, respectively) and fructose-2,6-biphosphate (21.0 ± 1.8 and 18.9 ± 2.0 nmol/g in xylitol+ and xylitol−, respectively) were not significantly different at the end of the xylitol and vehicle infusions. As shown in Fig. 7, the infusion of xylitol resulted in

![Graph](image-url)
marked increases in the rates of HGP, TGO, and glucose cycling. These results suggested that a moderate increase in the hepatic xylulose-5-P levels results in a marked increase in the in vivo flux through Glc-6-Pase.

Xylitol Infusion Reproduces the Effect of Glucose on Glc-6-Pase and PEPCK Gene Expression in Conscious Rats—Fig. 8 shows that increasing the hepatic concentration of xylulose-5-P by ~3-fold markedly increased the abundance of Glc-6-Pase mRNA in the liver of nondiabetic rats. This effect was quantitatively similar to that observed in the presence of a 2.5-fold increase in the plasma glucose concentration. Xylitol infusion also led to a marked decrease in the hepatic abundance of PEPCK mRNA. A significant increase (2–3-fold) in the hepatic concentration of xylulose-5-P (to 43 ± 12 nmol/g; p < 0.01) was also observed at the end of hyperglycemic clamp studies similar to those described in protocol 1. The similarities of the effects of xylitol and glucose on these two genes and the physiologic relevance of the observed increases in the hepatic concentration of xylulose-5-P (39, 40, 44, 45) provide strong evidence in support of a common mechanism mediating the actions of these substrates on Glc-6-Pase and PEPCK gene expression in the liver.

DISCUSSION

We provide direct evidence for a marked and rapid in vivo regulation of the hepatic levels of Glc-6-Pase mRNA and protein by glucose in conscious rats. Using a combination of the hyperglycemic and pancreatic clamp techniques, a glycemic target was achieved while carefully controlling other major hormonal and metabolic variables. The induction of hyperglycemia in conscious rats increased the level of Glc-6-Pase gene expression by >5-fold. The increase in Glc-6-Pase mRNA abundance was paralleled by an increase in Glc-6-Pase protein levels in vivo. Thus, we were able to demonstrate that the regulation of the gene expression of Glc-6-Pase by glucose occurs in conscious nondiabetic animals. In vivo this effect was observed within 1 h after induction of hyperglycemia and it achieved a plateau between 3 and 5 h. The plasma glucose concentration which elicited the induction of the hepatic Glc-6-Pase gene in the present study was ~17 mM, which is physiologically relevant in the context of poorly controlled diabetes or following a meal, when the portal glucose concentration is in this range. This stimulation was specific for Glc-6-Pase since the same experimental conditions, PEPCK mRNA abundance was markedly diminished by hyperglycemia, consistent with the notion that this gene is under negative control by the hexose (26). A moderate decrease (~40%) in Glk mRNA abundance was also observed following 3 and 5 h of hyperglycemia.

Glucose must be metabolized to modulate the gene expression of these enzymes and the mechanism by which it stimu-
lates liver Glc-6-Pase expression appeared to involve an intracellular metabolite resulting from hepatic glucose metabolism. We used an in vivo experimental approach to generate support for this hypothesis. Glucosamine is a potent inhibitor of the activity of GK (37, 42, 43), the enzyme which catalyzes the rate-limiting step for the hepatic utilization of glucose. This amino sugar was infused during hyperglycemic clamp studies to achieve plasma concentrations capable of markedly inhibiting the hepatic activity of GK. This maneuver almost completely abolished the effects of hyperglycemia on Glc-6-Pase and PEPCK gene expression in conscious rats. Taken together, these data indicated that glucose phosphorylation is a prerequisite for the effect of glucose on hepatic Glc-6-Pase expression and that the signal is generated by glucose metabolism downstream of Glc-6-P.

Several glycolytic, gluconeogenic, and lipogenic enzymes are controlled by carbohydrates at the transcriptional level (23–26, 28, 46). Carbohydrate responsive elements have been identified in some of these genes (27, 47). As we observed for the Glc-6-Pase gene in the present study, this regulation generally requires the phosphorylation of glucose (22, 28, 46). While the metabolic pathway of hepatic glucose utilization which regulates the transcription of most of these genes remains to be delineated, Doiron et al. (33) recently reported that the transcriptional regulation of the α-type pyruvate kinase gene by carbohydrates is mediated by a signal generated via the pentose phosphate pathway.

Since increasing the extracellular glucose concentrations in isolated cell systems or feeding a high carbohydrate diet in vivo increases the hepatic levels of xylulose-5-P by severalfold (39, 40, 44, 45, 48), we next examined whether a similar increase in the tissue levels of this metabolite, induced by means of an infusion of xylitol, would be sufficient to replicate the effects of hyperglycemia on hepatic Glc-6-Pase and PEPCK gene expression. In fact, xylulose-5-P is an intermediate in the nonoxidative branch of the pentose phosphate shunt which has been proposed to function as a sensor for hepatic glucose fluxes and to regulate the activity of fructose-6-phosphate 2-kinase/fructose-2,6-bisphosphatase (44, 45) and the transcription of the α-type pyruvate kinase (33) in the liver. During euglycemic-pancreatic clamp studies the infusion of xylitol, would be sufficient to replicate the effects of hyperglycemia on hepatic Glc-6-Pase and PEPCK gene expression. In fact, xylulose-5-P is an intermediate in the nonoxidative branch of the pentose phosphate shunt which has been proposed to function as a sensor for hepatic glucose fluxes and to regulate the activity of fructose-6-phosphate 2-kinase/fructose-2,6-bisphosphatase (44, 45) and the transcription of the α-type pyruvate kinase (33) in the liver. During euglycemic-pancreatic clamp studies the infusion of xylitol, would be sufficient to replicate the effects of hyperglycemia on hepatic Glc-6-Pase and PEPCK gene expression. 

FIG. 7. Effect of xylitol infusion on the rates of hepatic glucose production (A), total glucose output (B), and glucose cycling (C) during the pancreatic clamp studies. Rates of HGP were assessed under steady-state conditions during the basal period (Basal) and during the 3 h euglycemic-pancreatic clamp studies (3 h). Measurements were performed during the last 60 min of the basal period and clamp studies.

FIG. 8. Effect of xylitol infusion on the hepatic levels of Glc-6-Pase and PEPCK mRNA in conscious rats. A, Northern analysis of Glc-6-Pase mRNA was performed in liver freeze-clamped in situ at the completion of the euglycemic-pancreatic clamp studies. The plasma insulin concentration was maintained at basal levels in all studies. Total RNA (20 μg) of each liver RNA sample was loaded on each lane, equal loading was confirmed by ethidium staining of the 18 S and 28 S ribosomal RNA bands. The blots were probed with 32P-labeled Glc-6-Pase cDNA. The figure depicts Glc-6-Pase mRNA in groups (n = 2–3/each) of samples obtained from euglycemic control rats (xylitol−), rats in which vehicle was infused for 3 h, and rats in which xylitol (30 μmol/kg/min) was infused for 3 h (xylitol+). The bar graph depicts the average of quantitative analysis of at least 3 Northern blots. B, this figure shows Northern analysis of PEPCK mRNA abundance in liver freeze-clamped in situ at the completion of the in vivo studies. Total RNA (20 μg) of each liver RNA sample were loaded per lane. Equal loading was confirmed by ethidium staining of the 18 S and 28 S ribosomal RNA bands. Blots were probed with 32P-labeled PEPCK cDNA. The figure depicts PEPCK mRNA in groups (n = 2–3/each) of samples obtained from euglycemic control rats (xylitol−), rats in which vehicle was infused for 3 h, and rats in which xylitol (30 μmol/kg/min) was infused for 3 h (xylitol+). The bar graph depicts the average of quantitative analysis of at least 3 Northern blots.
crease in xylulose-5-P concentration did not induce sizeable changes in the levels of fructose-2,6-biphosphate. The use of postabsorptive rather than starred rats in the present studies may account for the lack of increase in fructose-2,6-biphosphate levels in the liver (39, 40, 48). While the dramatic effect of glucose and xylitol on Glc-6-Pase and PEPCK mRNA levels and on hepatic glucose fluxes strongly suggest a common mechanism for their action, our findings do not exclude the possibility that increased concentration of fructose-2,6-biphosphate could also play a role in the regulation of the Glc-6-Pase gene, particularly in the starved liver. A lag time was observed between the increase in Glc-6-Pase gene expression and changes in the in vivo glucose fluxes. This may reflect the time required for the synthesis and intracellular targeting of an active catalytic unit of the enzyme.

It may appear to be paradoxical that hyperglycemia increases Glc-6-Pase gene expression since the latter effect would favor further release of glucose by the liver. However, it should be noted that insulin, which increases concomitantly with glucose during meal absorption, has a potent inhibitory effect on the transcription of the Glc-6-Pase gene (8, 16, 18, 19, 21). The ability of hyperglycemia and other nutrients (36) to balance the transcription of the Glc-6-Pase gene (8, 16, 18, 19, 21). The expression of most hepatic enzymes in the same direction (21, 30, 32).

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