Mechanism by Which Hyperglycemia Inhibits Hepatic Glucose Production in Conscious Rats

Implications for the Pathophysiology of Fasting Hyperglycemia in Diabetes

Luciano Rossetti, Andrea Giaccari, Nir Barzilai, Kathleen Howard, Gary Sebel, and Meizhu Hu

Division of Endocrinology, Department of Medicine, Albert Einstein College of Medicine, Bronx, New York 10461; and Diabetes Division, Department of Medicine, University of Texas Health Science Center, San Antonio, Texas 78284

Abstract

To examine the relationship between the plasma glucose concentration (PG) and the pathways of hepatic glucose production (HGP), five groups of conscious rats were studied after a 6-h fast: (*a*) control rats (PG = 8.0±0.2 mM); (*b*) control rats (PG = 7.9±0.2 mM) with somatostatin and insulin replaced at the basal level; (*c*) control rats (PG = 18.1±0.2 mM) with somatostatin, insulin replaced at the basal level, and glucose infused to acutely raise plasma glucose by 10 mM; (*d*) control rats (PG = 18.0±0.2 mM) with somatostatin and glucose infusions to acutely reproduce the metabolic conditions of diabetic rats, i.e., hyperglycemia and moderate hypoinsulinemia; (*e*) diabetic rats (PG = 18.4±2.3 mM). All rats received an infusion of [3-^{3}H]glucose and [U-^{14}C]lactate. The ratio between hepatic [^{14}C]UDP-glucose sp act (SA) and 2× [^{14}C]-phosphoenolpyruvate (PEP) SA (the former reflecting glucose-6-phosphate SA) measured the portion of total glucose output derived from PEP-gluconeogenesis. In control rats, HGP was decreased by 58% in hyperglycemic compared to euglycemic conditions (4.5±0.3 vs. 10.6±0.2 mg/kg·min; $P < 0.01$). When evaluated under identical glycemic conditions, HGP was significantly increased in diabetic rats (18.9±1.4 vs. 6.2±0.4 mg/kg·min; $P < 0.01$). In control rats, hyperglycemia increased glucose cycling (by 2.5-fold) and the contribution of gluconeogenesis to HGP (91% vs. 45%), while decreasing that of glycogenolysis (9% vs. 55%). Under identical plasma glucose and insulin concentrations, glucose cycling in diabetic rats was decreased (by 21%) and the percent contribution of gluconeogenesis to HGP (73%) was similar to that of controls (84%). These data indicate that: (*a*) hyperglycemia causes a marked inhibition of HGP mainly through the suppression of glycogenolysis and the increase in glucokinase flux, with no apparent changes in the fluxes through gluconeogenesis and glucose-6-phosphatase; under similar hyperglycemic hypoinsulinemic conditions: (*b*) HGP is markedly increased in diabetic rats; however, (*c*) the contribution of glycogenolysis and gluconeogenesis to HGP is similar to control animals. (*J. Clin. Invest.* 1993, 92:1126–1134.) Key words: diabetes mellitus • glycogenolysis • glucokinase • gluconeogenesis • glucose cycling • glucose-6-phosphatase

Address reprint requests to Dr. Luciano Rossetti, Division of Endocrinology, Department of Medicine, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461.

Received for publication 12 January 1993 and in revised form 9 April 1993.

J. Clin. Invest.
© The American Society for Clinical Investigation, Inc.
0021-9738/93/09/1126/09 $2.00
Volume 92, September 1993, 1126–1134

Introduction

Overproduction of glucose is the major cause of fasting hyperglycemia in both insulin-dependent (1) and non–insulin-dependent diabetes mellitus (NIDDM) (2).[1] Gluconeogenesis composes an higher percentage of hepatic glucose production (HGP) in diabetic as compared with nondiabetic individuals (3–6), and it has been postulated to be the "primary" determinant of increased HGP in diabetes (5). However, recent experimental evidence suggests that changes in the rates of formation of hepatic glucose-6-phosphate (G6P) through gluconeogenesis do not alter HGP in conscious dogs (7), healthy volunteers (8–10), and NIDDM subjects (11). The "final common pathway" for the net release of glucose into the circulation is regulated by the balance of fluxes through glucokinase and glucose-6-phosphatase (G6Pase). The assessment of in vivo metabolic fluxes contributing to G6P formation and to hepatic glucose output, in combination with the steady-state changes in the hepatic G6P concentration, may allow one to discern if the key regulatory site for HGP is the formation of G6P or its net dephosphorylation to glucose. See Fig. 1 for a schematic representation.

Soskin and Levine (12) first proposed that mammalian liver can rapidly change its glucose output in response to changes in the circulating glucose concentration independently from hormonal signals. It is now recognized that the plasma glucose concentration per se regulates HGP (13–18) and as much as 50% of the decline in plasma glucose concentration after glucose administration may be due to the combined effect of hyperglycemia per se on glucose disposal and HGP (18). However, relatively little information is available about the mechanism(s) by which hyperglycemia, independent of insulin, inhibits HGP. Basal HGP is markedly increased in some diabetic states despite the presence of hyperglycemia and normo- or hyperinsulinemia, both of which are known to suppress HGP (2, 13–18). This may suggest a role for defective glucose-induced suppression of HGP in the pathophysiology of fasting hyperglycemia in diabetes.

HGP is composed of glycogen mobilization and gluconeogenesis. However, under postabsorptive conditions, i.e., basal insulin, a sizeable portion of the flux through G6Pase also comes from glucose cycling (19–23). Although hyperglycemia per se inhibits HGP, such an effect has not been demonstrated for total glucose output (TGO) (21), i.e., flux through G6Pase. Thus, it is possible that a component of the effect of hyperglycemia per se in suppressing HGP is mediated by the enhanced

1. *Abbreviations used in this paper:* G6P, glucose-6-phosphate; G6Pase, glucose-6-phosphatase; GC, glucose cycling; HGP, hepatic glucose production; NIDDM, non-insulin-dependent diabetes mellitus; PEP, phosphoenolpyruvate; SRIF, somatostatin; TGO, total glucose output.

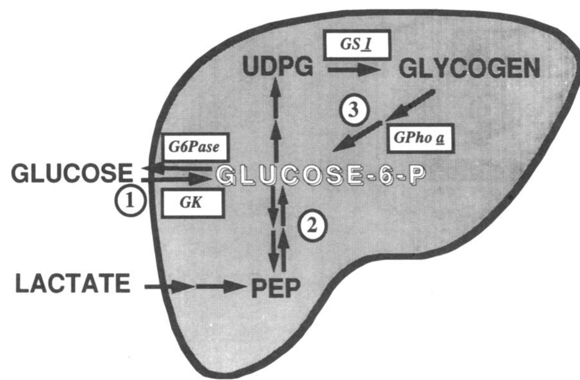

Figure 1. Schematic representation of the major patways and enzymatic steps which regulate hepatic glucose production. In a net sense, the hepatic glucose-6-phosphate (*glucose-6-P*) pool receives three major inputs: (*a*) plasma-derived glucose, (*b*) gluconeogenesis, and (*c*) glycogenolysis. The final common pathway for hepatic glucose output is the net dephosphorylation of G6P, which is regulated by the balance of glucokinase (*GK*) and G6Pase activities. Similarly, the net contribution of hepatic glycogen to the G6P pool represents the balance of the fluxes through glycogen synthase and glycogen phosphorylase. The relative contribution of plasma glucose and gluconeogenesis to the hepatic G6P pool can be directly measured by tracer methodology. In fact, after [3-^{3}H]glucose infusion, the ratio of specific activities of tritiated hepatic UDP-glucose (*UDPG*) and plasma glucose represents the percentage of the hepatic G6P pool which is derived from plasma glucose. Similarly, after [U-^{14}C]lactate infusion, the proportion of the G6P pool which is formed through PEP-gluconeogenesis can be calculated as the ratio of ^{14}C-labeled UDP-glucose and PEP.

phosphorylation of plasma glucose, i.e., increased flux through glucokinase. In that a marked decrease in hepatic glucokinase activity has been shown in several animal models of diabetes mellitus (24), an impairment in the hepatic capacity to phosphorylate glucose may cause a blunted glucose-induced rise in glucose cycling and a diminished inhibition of HGP.

The short-term in vivo regulation of glucokinase and G6Pase fluxes and activity remains controversial. In particular, the mechanism(s) by which acute changes in the plasma glucose concentration cause alterations in HGP have not been delineated. Because several hepatic enzymes are defective in the diabetic liver, the combined assessment of in vivo glucose fluxes, substrate concentrations, and in vitro enzyme activities is required to distinguish the relative role of these biochemical alterations on in vivo hepatic glucose metabolism. Our results suggest that (*a*) acute hyperglycemia (in the presence of low insulin) causes a marked inhibition of HGP in normal rats mainly through the suppression of glycogenolysis and through a marked increase in glucokinase flux (increased glucose cycling), with no apparent changes in the fluxes through gluconeogenesis and G6Pase and (*b*) under similar conditions of hyperglycemia and hypoinsulinemia, increased glycogenolysis and gluconeogenesis and decreased glucose cycling (GC) all contribute to the enhanced HGP in diabetic rats.

Methods

Animals. Two groups of male Sprague-Dawley rats (Charles River Breeding Laboratories, Inc., Wilmington, MA) were studied: group I, controls ($n = 44$); group II, partially pancreatectomized rats ($n = 10$). At 3–4 wk of age, all rats (80–100 g) were anesthetized with pentobarbital (50 mg/kg body wt i.p.) and in group II 90% of their pancreas was removed according to the technique of Foglia (25), as modified by Bonner-Weir et al. (26). Immediately after surgery rats were housed in individual cages and subjected to a standard light (6 a.m. to 6 p.m.)/dark (6 p.m. to 6 a.m.) cycle. After surgery rats were weighed twice weekly and tail vein blood was collected for the determination of non-fasting plasma glucose and insulin concentrations at the same time (8 a.m.). The fasting plasma glucose and insulin concentrations also were determined weekly on tail vein blood. 5 wk after pancreatectomy rats were anesthetized with 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, as previously described (23, 27, 28).

Insulin/somatostatin/infusions. Studies were performed in awake, unstressed, chronically catheterized rats using the euglycemic or hyperglycemic clamp technique in combination with [2-^{3}H]glucose or [3-^{3}H]glucose infusions as previously described (23, 27, 28). Rats were fasted for 6 h before the in vivo studies. Briefly, a prime-continuous infusion of somatostatin (0.8 μg/kg · min) and regular insulin ($\sim$ 0.4 mU/kg · min) or saline (protocols 1 and 4) were administered, and a variable infusion of a 25% glucose solution was started at time zero and periodically adjusted to clamp the plasma glucose concentration at $\sim$ 8 mM (euglycemic studies; protocol 2) or $\sim$ 18 mM (hyperglycemic studies; protocol 3A and 3B).

During the euglycemic study the insulin infusion was adjusted to maintain normoglycemia during the somatostatin infusion. The average insulin infusion required to maintain normoglycemia in control rats was 0.4±0.1 mU/kg · min, without need for glucose infusion. 80 min before starting the insulin/somatostatin infusion, a prime-continuous infusion of [^{3}H-3]glucose (New England Nuclear, Boston, MA; 40 μCi bolus, 0.4 μCi/min) was initiated and maintained throughout the basal period and stopped before initiating the somatostatin/insulin infusions. At $t = 30$ min into the infusion study (a time when the tritiated glucose infused during the basal period was not detectable in plasma and a new "metabolic" steady-state was achieved) a prime-continuous infusion of [3-^{3}H]glucose (15–40 μCi bolus, 0.4 μCi/min) was initiated and maintained throughout the remainder of the study. [U-^{14}C]-lactate (20 μCi bolus/1.0 μCi/min) was infused during the last 10 min of the study. A subgroup of nine 6-h fasted rats received an hyperglycemic clamp study in combination with somatostatin infusion (as in protocol 3B), but [2-^{3}H]glucose rather than [3-^{3}H]glucose was used to measure the rate of glucose turnover. Plasma samples for determination of [^{3}H]glucose specific activity were obtained at 10-min intervals throughout the basal period and the insulin/somatostatin infusions. Plasma samples for determination of plasma insulin and glucagon concentrations were obtained at time −30, 0, 30, 60, 90, 120 min during the study. The total volume of blood withdrawn was $\sim$ 3.0 ml per study; to prevent volume depletion and anemia, a solution (1:1 vol/vol) of $\sim$ 4.0 ml of fresh blood (obtained by heart puncture from a littermate of the test animal) and heparinized saline (10 U/ml) was infused. All determinations were also performed on portal vein blood obtained at the end of the experiment. At the end of the insulin infusion, rats were anesthetized (pentobarbital 60 mg/kg body wt, i.v.), the abdomen was quickly opened, portal vein blood obtained and liver was 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 < 45 s. All tissue samples were stored at −80°C for subsequent analysis.

The study protocol was reviewed and approved by the Institutional Animal Care and Use Committees of the University of Texas Health Science Center at San Antonio and of the Albert Einstein College of Medicine.

Glycogen synthase. Hepatic glycogen synthase activity was measured by a modification (29, 30) of the method of Thomas et al. (31) and is based on the measurement of the incorporation of radioactivity into glycogen from UDP-[U-^{14}C]glucose. Tissue samples (20–30 mg) were homogenized in 2.0 ml of Tris/HCl buffer, pH 7.8, containing 10 mM EDTA, 5 mM dithiothreitol, 50 mM NaF, and 2.5 g/liter rabbit

liver glycogen type III. The homogenate was centrifuged at 2,000 g for 15 min (at 4°C) and the supernatant used for glycogen synthase assay by measuring the incorporation of UDP-[U-^{14}C]glucose into glycogen at 30°C. Synthase I activity is defined as that activity assayable in the presence of 0.11 mM G6P. In order to approximate the in vivo glycogenic rates incubations are also carried out in presence of the UDP-glucose concentrations (125–500 μM) within the physiological range for rat liver. Total glycogen synthase D activity is measured in the presence of 7.2 mM G6P. The percentage of synthase in the active ($FV_{0.1}$) form is defined as the ratio of activities (I/D) $\times$ 100.

Glycogen phosphorylase. Liver glycogen phosphorylase activity was measured as previously described (32). This assay is based on the measurement of the incorporation of ^{14}C into glycogen from labeled glucose-1-phosphate. Glycogen phosphorlase *a*, the active phosphorylated enzyme, was assayed in the absence of AMP. Tissue homogenates (20–30 mg) were prepared as described above. The supernatant was used for glycogen phosphorylase assay by measuring the incorporation of [^{14}C]glucose-1-phosphate into glycogen at 30°C in a mixture containing 33 mM Mes, 200 mM KF, 0.45% mercaptoethanol, 15 mM glucose-1-phosphate (50 μCi/mmol), and 3.4 mg/ml glycogen.

Glucokinase. Hepatic glucokinase activity was measured by the continuous assay spectrophotometric method as described by Davidson and Arion (33), with some modifications. Liver homogenates ($\sim$ 200 mg) were prepared in 50 mM Hepes, 100 mM KCl, 1 mM EDTA, 5 mM $MgCl_2$, and 2.5 mM dithioerythreitol. Homogenates were centrifuged at 100,000 g for 45 min to sediment the microsomal fraction (which will be used for the G6Pase assay). The postmicrosomal fraction is assayed in a medium, pH 7.4 at 37°C, containing 50 mM Hepes, 100 mM KCl, 7.5 mM $MgCl_2$, 5 mM ATP, 2.5 mM dithioerythreitol, 10 mg/ml albumin, and 0.5 mM (exokinases activity) or 7, 18, and 100 mM glucose (total phosphorylating activity), 0.5 mM NAD^+, 4 U of G6P dehydrogenase (*L. mesenteroides*) and the equivalent of $\sim$ 1 mg of wet liver. The reaction is initiated by the addition of ATP, and the rate of NAD^+ reduction will be recorded at 340 nm. Glucose phosphorylation is determined as the absorbance change in the complete medium minus the absorbance change in the absence of ATP under conditions in which the absorbance is increasing linearly with time (generally from 15 to 40 min). This improved assay procedure provides an accurate measure of the hepatic glucose phosphorylating capacity and, in the presence of physiological glucose concentrations (7–18 mM), allow one to approximate the in vivo glucokinase activity (33).

Glucose-6-phosphatase. The assay was performed as described by Burchell et al. (34) and is based on the dissociation of P_i from G6P by the tissue microsomal fraction containing the G6Pase. The microsomal fraction was incubated with 1 mM G6P. The reaction was stopped after 20 min with solution containing acid molybdate, with 2/9 vol of 10% SDS and 1/9 vol of 10% ascorbic acid. It was then incubated for 20 min at 45°C, and the absorbance read at 820 nM. Standard curve is obtained from different concentrations of P_i.

Analytical procedures. Plasma glucose was measured by the glucose oxidase method (Glucose Analyzer II, Beckman Instruments, Inc., Palo Alto, CA) and plasma insulin by radioimmunoassay using rat and porcine insulin standards. Plasma [^{3}H]glucose radioactivity was measured in duplicate on the supernatants of $Ba(OH)_2$ and $ZnSO_4$ precipitates (Somogyi procedure) of plasma samples after evaporation to dryness to eliminate tritiated water. Liver G6P concentrations were measured spectrophotometrically as described by Michal (35). Liver glycogen concentration was determined as previously described (23, 27). Uridinediphosphoglucose (UDP-glucose) and phosphoenolpyruvate (PEP) concentrations and specific activities in the liver were obtained through two sequential chromatographic separations, as previously reported (23, 36). Differences between groups were determined by ANOVA analysis of variance.

Terminology. In the present manuscript the term "total glucose output" (TGO) is intended as total in vivo flux through G6Pase as measured by [2-^{3}H]glucose turnover, and the term "hepatic glucose production" (HGP) is intended as the net rates of G6P dephosphorylation to glucose (balance of the in vivo fluxes through glucokinase and G6Pase) as measured by [3-^{3}H]glucose turnover. Finally, "glucose cycling (GC) is defined as imput of extracellular glucose into the G6P pool followed by exit of plasma-derived G6P back into the extracellular pool.

Calculations. The HGP was calculated as the difference between the tracer-derived rate of appearance and the infusion rate of glucose. Gluconeogenesis (G) was estimated from the specific activities (SA) of ^{14}C-labeled hepatic UDP-glucose (this is assumed to reflect the specific activity of hepatic G6P), and hepatic PEP after the infusion of [U-^{14}C]-lactate and [3-^{3}H]glucose: G = TGO $\times$ [^{14}C]UDP-glucose SA/[^{14}C]-PEP SA $\times$ 2.

Glycogenolysis was calculated as the difference between the HGP and the gluconeogenesis. An additional estimate of hepatic glycogenolysis was obtained from the decrease in liver glycogen concentration (below the basal values obtained during the saline infusions) during the in vivo study.

The percentage of the hepatic G6P pool directly derived from plasma glucose can be calculated as the ratio of [^{3}H]UDP-glucose and plasma [3-^{3}H]glucose specific activities. Thus, this ratio also measures the percent contribution of plasma glucose to the G6Pase flux (i.e., GC). Since TGO is equal to the sum of the HGP + GC (and GC = [^{3}H]UDP-glucose SA/plasma [3-^{3}H]glucose SA $\times$ TGO), the equation can be resolved to calculate both GC and TGO: TGO = HGP/(1 − [^{3}H]UDP-glucose SA/plasma [3-^{3}H]glucose SA) and GC = [^{3}H]UDP-glucose SA/plasma [3-^{3}H]glucose SA $\times$ TGO (23).

The above calculations are based on the assumptions that (*a*) the specific activity of hepatic UDP-glucose reflects that of G6P and (*b*) that complete detritiation of the [3-^{3}H]glucose is completed at the level of the triose-phosphate pool. In separate studies in 6-h fasted conscious rats we have verified the correspondence of ^{14}C specific activities of the hepatic UDP-glucose and G6P after the infusion of [U-^{14}C]-lactate. The absence of tritiated label at the level of the hepatic PEP was confirmed in all the studies, indicating that all of the ^{3}H label is lost in the metabolism of the [3-^{3}H]glucose through glycolysis. However, a small percentage of [3-^{3}H]glucose may also enter the pentose phosphate pathway, undergo detritiation at the ribulose-5-phosphate level, and reenter in the G6P pool. In fact, for each molecule of [3-^{3}H]-glucose that enters the pentose phosphate pathway (generating 3 CO_2 and 1 glyceraldehyde-3-phosphate), about two other detritiated molecules of [3-^{3}H]G6P may reenter the G6P pool and therefore dilute its specific activity. Thus, this tracer will determine an underestimation of the glucose cycling which is in direct proportion to the rate of the pentose phosphate cycle (23). Similarly, if there was significant fructose-6-phosphate cycling during the present studies, the use of [3-^{3}H]-glucose (rather than [6-^{3}H]glucose) would cause an underestimation of the rate of GC and an overestimation of HGP. Although we have previously shown similar relative UDP-glucose specific activities after [3-^{3}H]glucose or [6-^{3}H]glucose infusions under hyperinsulinemic and hyperglycemic conditions (23), this was not verified under the present experimental conditions. It should also be pointed out that this tracer methodology will measure PEP-gluconeogenesis, which represents the great majority of the gluconeogenic flux under most experi-

Table I. General Characteristics of Control and Diabetic Rats

Group	Control	Diabetic
n	35	10
Body wt (*g*)	322±7	305±12
Fasting plasma glucose (*mM*)	5.6±0.2	7.2±0.4*
Fasting plasma insulin (*μU/ml*)	26±3	20±6
Nonfasting plasma glucose (*mM*)	7.7±0.4	18.5±1.3*
Nonfasting plasma insulin (*μU/ml*)	51±3	26±5*

* $P < 0.01$ vs. control.

Table II. Steady-State Plasma Glucose, Insulin, and Glucagon Concentrations During the In Vivo Studies

Group	Glucose	Insulin	Glucagon
	mM	*μU/ml*	*pg/ml*
1. Control (C)	8.0±0.2	46±5	118±11
2. C/euglycemic	7.9±0.2	43±3	115±14
3A. C/hyperglycemic	18.1±0.2*	45±3	103±10
3B. C/hyperglycemic	18.0±0.2*	24±4*	106±19
4. 90% pancreatectomized rats	18.4±2.3*	27±6*	119±16

* $P < 0.01$ vs. 2-C/euglycemic.

mental conditions. However, it may significantly underestimate the overall gluconeogenic rate under experimental conditions in which non-PEP-gluconeogenesis is significantly increased.

Results

Basal metabolic parameters. There were no differences in the mean body weights between control and diabetic rats (Table I). Both the fasting ($P < 0.05$) and nonfasting ($P < 0.01$) plasma glucose concentrations during the 2-wk period before the in vivo studies were significantly elevated in the diabetic compared to the control group (Table I). The fasting plasma insulin concentrations were similar, while the nonfasting plasma insulin concentrations were significantly decreased in diabetic ($P < 0.01$).

Effect of hyperglycemia on hepatic glucose fluxes and hepatic substrate levels and enzyme activities in control rats (study protocols 1, 2, and 3A; Tables II–V; Figs. 2 and 3). The steady-state plasma glucose concentration was kept at the basal level (~ 8 mM) throughout the study protocols 1 and 2, while it was raised by ~ 10 mM during the study protocols 3A and B. In studies 2 and 3A the plasma insulin concentration was maintained at the levels measured during study protocol 1, using combined somatostatin and insulin infusions. In study 3B the infusion of somatostatin led to a decrease in the plasma insulin concentration to a level similar to that of diabetic rats (Table II). Plasma glucagon concentration was similar in the experimental groups (Table II).

Table III displays the [^{3}H]- and [^{14}C]UDP-glucose specific activities, the [^{14}C]PEP and the [^{3}H]glucose specific activities which are used to calculate the contribution of plasma-derived glucose ("Direct" in Table III) and the contribution of PEP-derived G6P ("Indirect" in Table III) to the hepatic G6P pool. The UDP-galactose specific activities confirmed the values obtained with UDP-glucose, suggesting rapid and complete isotopic equilibration between the two intracellular pools.

HGP was similar in the two euglycemic studies (10.6±0.6 and 10.6±0.2 mg/kg · min in protocols 1 and 2, respectively), but it was significantly suppressed by hyperglycemia, in the presence of basal insulin (4.5±0.3 vs. 10.6±0.2 mg/kg · min, $P < 0.01$; protocol 3A vs. protocol 2; Fig. 2 *A*) and with hypoinsulinemia (6.2±0.4 mg/kg · min, $P < 0.01$; protocol 3B vs. protocol 2). TGO (G6Pase flux; Fig. 2 *B*) was similar in the presence of normoglycemia (protocol 1 = 13.4±0.6 mg/kg · min and 2 = 14.2±0.7 mg/kg · min) or hyperglycemia (protocol 3A = 13.5±0.5 mg/kg · min; P = NS). Under basal insulin conditions, glucose cycling (Fig. 2; *C*) was markedly enhanced by hyperglycemia (9.0±0.6 vs. 3.6±0.6 mg/kg · min, $P < 0.01$ protocol 3A vs. 2). Under basal insulin conditions, hyperglycemia led to a decrease in the hepatic G6P and UDP-glucose concentrations (Tables IV and V). The reduction in the hepatic G6P concentration was significant between protocols 3A and 1, but did not reach statistical significance between groups 3A and 2. Fig. 3 displays the contribution of hepatic gluconeogenesis (*A*) and glycogenolysis (*B*) to HGP in the presence of normo- or hyperglycemia and basal insulinemia. Hyperglycemia led to a marked reduction in hepatic glycogenolysis (0.4±0.2 vs. 5.8±0.5 mg/kg · min, $P < 0.01$ study 3A vs. 2), whereas gluconeogenesis was only slightly decreased (4.0±0.5 vs. 4.8±0.5 mg/kg · min, $P = 0.07$ study 3A vs. 2). Consistent with this finding, the residual hepatic glycogen at the end of the infusion studies was greater in the hyperglycemic than in the normoglycemic studies (Table IV; $P < 0.01$ study 3A vs. 2). The effect of the plasma glucose concentration on hepatic glycogen synthase and phosphorylase activities is displayed in Table IV. $FV_{0.1}$ was measured as the mean of the values obtained at three UDP-glucose concentrations within the physiological range for rat liver (36), i.e., 0.125, 0.250, and 0.500 mM. Under basal insulin conditions, hyperglycemia activated the hepatic glycogen synthase and decreased the activity of the glycogen phosphorylase *a* ($P < 0.01$ study 3A vs. 2). Hepatic glucokinase and G6Pase activities were unchanged by short-term hyperglycemia (Table V).

Comparison of control and diabetic rats under similar hypoinsulinemic and hyperglycemic conditions (study protocols 3B and 4; Figs. 4 and 5). HGP (18.9±1.4 vs. 6.2±0.4 mg/

Table III. Substrate Specific Activities at the End of the [3-^{3}H]Glucose-[U-^{14}C]Lactate Infusion

Group	[^{14}C]PEP	[^{14}C]UDPGlu	[^{14}C]UDPGal	Indirect		[^{3}H]Glu	[^{3}H]UDPGlu	[^{3}H]UDPGal	Direct	
				UDPGlu	UDPGal				UDPGlu	UDPGal
		dpm/nmol		*%*			*dpm/nmol*		*%*	
1. Control (C)	9.9±1.9	7.9±1.8	7.7±2.1	38.7±3.1	37.9±3.4	41.5±5.8	9.0±1.3	9.5±1.4	20.9±1.2	22.7±1.2
2. C/euglycemic	11.3±1.4	7.6±1.0	7.6±1.2	34.0±2.2	34.3±2.7	39.5±3.9	9.9±0.9	10.2±1.3	25.4±1.6	25.7±1.8
3. C/hyperglycemic	9.7±1.3	6.5±1.1	6.4±0.8	33.0±3.3	33.4±3.4	14.3±1.3	9.0±1.1	9.3±1.0	60.7±2.7	62.0±3.0
4. PANX	10.1±1.9	10.8±2.5	10.6±3.0	52.5±4.8	51.4±5.0	15.7±1.4	4.5±0.3	4.8±0.5	28.9±2.0	29.4±1.5

Abbreviations: UDPGlu, uridinediphosphoglucose; UDPGal = uridinediphosphogalactose; Glu, plasma glucose; Direct = percentage of the hepatic G6P pool derived from plasma glucose, calculated as the ratio of the specific activities of [^{3}H]UDPGlu (Glu) or [^{3}H]UDPGal (Gal) and [^{3}H]Glu; Indirect = percentage of the hepatic G6P pool derived from PEP-gluconeogenesis, calculated as the ratio of the specific activities of [^{14}C]UDPGlu (Glu) or [^{14}C]UDPGal (Gal) and [2-^{14}C]-PEP.

kg·min; $P < 0.01$ protocol 4 vs. 3B; Fig. 4 *A*) and TGO (26.5±1.5 vs. 15.8±0.9 mg/kg·min; $P < 0.01$, group 4 vs. 3B; Fig. 4 *B*) were markedly increased in diabetic compared to control rats. In the presence of similar plasma insulin and glucose concentrations, GC (Fig. 4 *C*) was significantly less in diabetic animals (7.6±0.7 vs. 9.6±0.7 mg/kg·min, $P < 0.01$ group 4 vs. 3B). The hepatic UDP-glucose concentration was similar in diabetic and control rats (Table IV), but the G6P concentration (Table V) was significantly lower in diabetic rats ($P < 0.01$, group 4 vs. 3B). Fig. 5 displays the contribution of hepatic gluconeogenesis (*A*) and glycogenolysis (*B*) to HGP. Both enhanced hepatic glycogenolysis (4.9±1.0 vs. 0.9±0.3 mg/kg·min, $P < 0.01$ group 4 vs. 3B) and gluconeogenesis (13.9±1.8 vs. 5.2±0.3 mg/kg·min, $P < 0.01$ study 4 vs. 3B) contributed to the marked increase in HGP in the diabetic rats. The TGO (16.0±1.1 mg/kg·min), the gluconeogenesis (5.6±0.4), and the GC (9.8 mg/kg·min) were similar in the subgroup of control rats, receiving protocol 3B in combination with [2-^{3}H]glucose rather than [3-^{3}H]glucose.

Table IV. Fractional Velocity (GS $FV_{0.1}$) of Hepatic Glycogen Synthase, Activity of AMP-independent (Pho a) Form of Hepatic Glycogen Phosphorylase, Hepatic Uridinediphosphoglucose (UDPGlu), and Glycogen Concentrations (Glycogen) at the End of the In Vivo Studies

Group	GS $FV_{0.1}$	Pho *a*	UDPGlu	Glycogen
	%	*μmol/g·min*	*nmol/g*	*μmol/g*
1. Control (C)	29.4±1.9	14.1±1.4	358±11	88±13
2. C/euglycemic	28.5±1.4	17.6±2.2	390±14	84±5
3A. C/hyperglycemic	40.2±1.8*	9.7±0.9*	259±10*	139±11*
3B. C/hyperglycemic	38.6±2.9*	11.3±0.7*	276±12*	130±9*
4. PANX	17.9±1.1‡	9.1±1.0*	279±24*	116±12*

The $FV_{0.1}$ for the hepatic glycogen synthase represent the mean of the activity ratios in the presence of three physiologic UDPGlu concentrations (0.125, 0.25, and 0.5 mM) with 0.11 or 7.2 mM G6P. The Pho *a* is the hepatic glycogen phosphorylase activity in the absence of AMP. * $P < 0.01$ vs. 2-C/euglycemic; ‡ $P < 0.01$ vs. 3B-C/hyperglycemic.

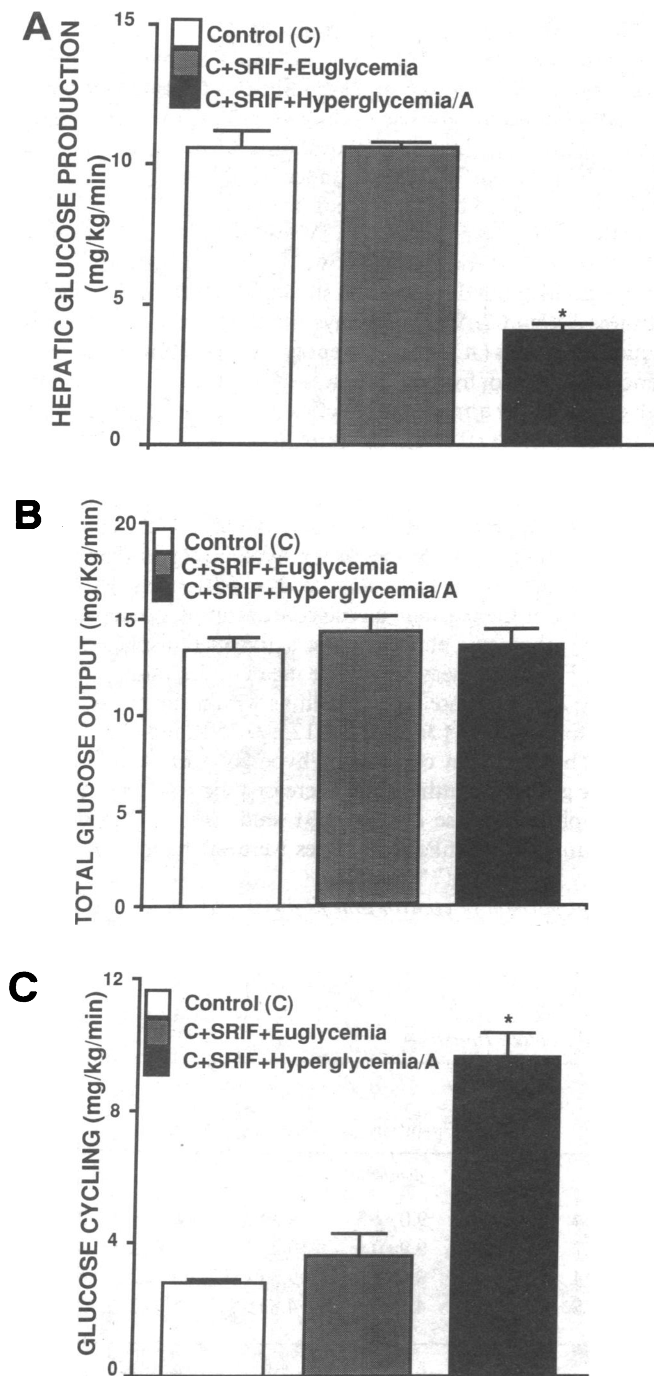

Figure 2. Effect of hyperglycemia, under basal insulin conditions, on (*A*) HGP, (*B*) TGO, and (*C*) GC in control rats. *$P < 0.01$ vs. C + somatostatin (SRIF) + euglycemia.

Despite lower plasma insulin concentrations, hyperglycemia activated the hepatic glycogen synthase and decreased the activity of the glycogen phosphorylase *a* (Table IV; $P < 0.01$ study 3B vs. 2) in control rats. The activity of the hepatic glycogen synthase was severely impaired in diabetic compared to control rats (Table IV). Hepatic glucokinase activity was markedly decreased (about twofold) and G6Pase activity increased (about twofold) in diabetic compared to control rats (Table V).

Correlations. In the control groups (I, II, IIIA and B), the HGP was inversely correlated to the rate of glucose cycling (Fig. 6, *left*), while there was no significant correlation with the total glucose output. In the control groups, the HGP was highly correlated to the rate of hepatic glycogenolysis, while a much weaker correlation was present between the HGP and the rate of gluconeogenesis (Fig. 6, *right*). In diabetic rats (group IV), the HGP was highly correlated to the TGO ($r^2 = 0.904$; $P < 0.01$) and to the rates of gluconeogenesis ($r^2 = 0.624$; $P < 0.05$), while there was no significant correlation between HGP and glucose cycling ($r^2 = 0.204$; P = NS) and glycogenolysis ($r^2 = 0.014$; P = NS).

Discussion

Postabsorptive HGP and GC are markedly elevated in hyperglycemic NIDDM individuals despite normal or elevated plasma insulin (2–5, 20). Because the enhanced contribution of gluconeogenesis has been shown to completely account for the increased HGP (3, 5), several investigators have suggested that the overactivity of the gluconeogenic pathway is the primary defect responsible for fasting hyperglycemia in NIDDM

Table V. *Activity of Hepatic Glucokinase (GK) and G6Pase and Hepatic G6P and Glucose (Glu) Concentration at the End of the In Vivo Studies*

Group	GK (7 mM)	GK (18 mM)	G6Pase	G6P	Glu
		μmol/g/min		*nmol/g*	*μmol/g*
1. Control (C)	3.8±0.5	6.3±0.7	4.2±0.7	564±62	7.8±0.2
2. C/euglycemic	4.0±0.4	6.4±0.8	4.7±0.5	522±28	7.5±0.3
3A. C/hyperglycemic	3.9±0.3	6.3±0.9	4.1±0.7	494±18	16.1±0.3*
3B. C/hyperglycemic	3.6±0.4	5.9±0.5	5.2±0.4	465±24*	15.9±0.2*
4. PANX	1.4±0.1*‡	2.3±0.1*‡	10.4±1.1*‡	342±23*‡	16.6±2.1*

The activity of the hepatic glucokinase is calculated in the presence of 7 or 18 mM glucose to approximate the in vivo plasma glucose concentration in the four experimental groups. The G6Pase activity is measured in the presence of a physiologic (1 mM) G6P concentration.
* $P < 0.01$ vs. 2-C/euglycemic; ‡ $P < 0.01$ vs. 3B-C/hyperglycemic.

(2, 3, 5). However, the comparison of diabetic and control subjects under different glycemic conditions appears to underestimate the potential role of the plasma glucose concentration per se in the regulation of the pathways of HGP.

Although a major role for the circulating plasma glucose concentration in the regulation of HGP has long been recognized (12–18), the mechanism(s) by which changes in glucose level per se inhibit HGP and the impact of the concomitant hyperglycemia in the interpretation of hepatic glucose fluxes in diabetes have not been delineated. Thus, the present study examined the mechanisms by which hyperglycemia regulates HGP in conscious rats.

The major metabolic steps which regulate HGP may be schematically divided into two categories (see Fig. 1): (*a*) formation of hepatic G6P; and (*b*) phosphorylation/de-phosphorylation of G6P. The hepatic G6P pool can derive form three major sources: gluconeogenesis, glycogenolysis and plasma glucose, i.e., GC. The two key enzymes which regulate the net dephosphorylation of the hepatic G6P to glucose are glucokinase and G6Pase. The relative contribution of plasma glucose and gluconeogenesis to the hepatic G6P pool can be directly measured by tracer methodology. In fact, the proportion of the G6P pool which is derived from plasma glucose can be calculated as the ratio of specific activities of tritiated hepatic UDP-glucose and plasma glucose after [3-^{3}H]glucose infusion and the percentage of the G6P pool which is formed through PEP-gluconeogenesis can be calculated as the ratio of ^{14}C-labeled UDP-glucose and PEP after [U-^{14}C]lactate infusion (23).

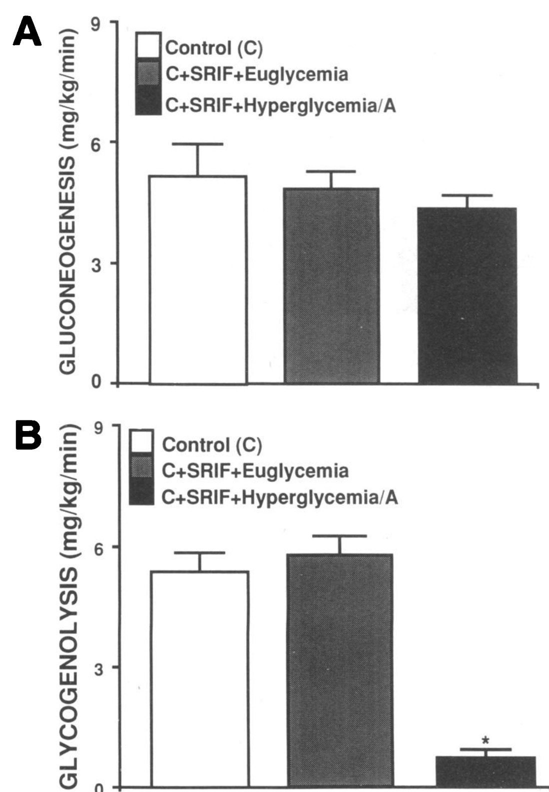

Figure 3. Effect of hyperglycemia, under basal insulin conditions, on the rates of (*A*) gluconeogenesis and (*B*) hepatic glycogenolysis. *$P < 0.01$ vs. C + SRIF + euglycemia.

To examine the effect of hyperglycemia, independent of hormonal signals, on hepatic glucose fluxes, we combined the infusion of somatostatin and insulin, with either euglycemia (~ 8 mM) or hyperglycemia (~ 18 mM). In the presence of euglycemia, the infusion of somatostatin and insulin did not cause any significant alteration in HGP, TGO, GC, gluconeogenesis, or glycogenolysis (Figs. 2 and 3). Hyperglycemia caused a 58% decrease in HGP compared to the euglycemic condition, while total G6Pase flux was not significantly decreased. This discrepancy between total and net glucose output was due to a 2.5-fold elevation in glucose cycling. Such an increase in the contribution of plasma-derived glucose carbons to the G6Pase flux was proportional to the ~ 2.3-fold increase in the circulating glucose concentration, suggesting that it is largely mediated through the increase in substrate for hepatic glucokinase. This interpretation of the in vivo fluxes is supported by the enzyme activities and substrate concentrations displayed in Table V. In fact, a 2.2-fold increase in the hepatic glucose concentration did not change the glucokinase and the G6Pase activities. However, the glucokinase activity measured in the presence of 18 mM glucose was increased by about two-fold compared to the enzyme's activity in the presence of 7 mM glucose, suggesting that the increased availability of substrate rather than an increase in enzyme activity accounted for the enhanced contribution of plasma-derived glucose to the G6P pool. Similarly, the lack of effect of acute hyperglycemia on G6Pase activity and hepatic G6P concentration is consistent with the lack of glucose-induced inhibition of in vivo total glucose output. The ability of hyperglycemia per se to suppress HGP was confirmed in a subgroup of rats in which insulin was not replaced during the hyperglycemic study. Despite the pres-

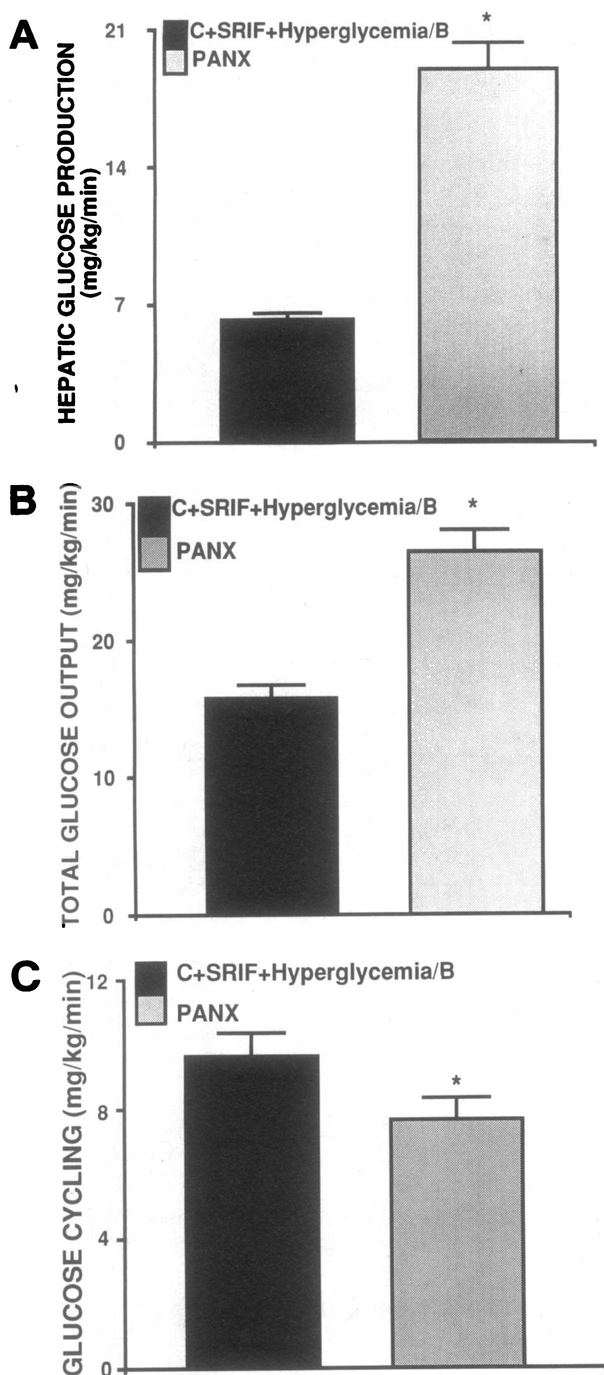

Figure 4. (*A*) HGP, (*B*) TGO, and (*C*) GC in control and 90% pancreatectomized diabetic rats, in the presence of similar conditions of hyperglycemia and moderate hypoinsulinemia (control rats received SRIF without insulin replacement; protocol 3B). $^*P < 0.01$ vs. C + SRIF + hyperglycemia/B.

ence of hypoinsulinemia, HGP was inhibited by 42% compared to euglycemic control. Hyperglycemia increased glucose cycling by 2.7-fold, and slightly (by 12%) TGO, similarly to what observed in the presence of basal insulin.

The decrease in HGP induced by acute hyperglycemia can be the consequence of diminished flux from glycogen, gluconeogenesis, or both. In the presence of basal insulin concentrations, the increase in circulating glucose caused an almost complete inhibition of hepatic glycogenolysis, which accounted for 89% of the decline in HGP. Although gluconeogenesis was also slightly diminished (16%) compared to the euglycemic control, this decline was inconsistently observed and did not reach statistical significance. Similarly, in the presence of hypoinsulinemia, the hyperglycemia-induced suppression of hepatic glycogenolysis accounted for all of the decrease in HGP, while gluconeogenesis was marginally increased (by 8%). Consistent with the in vivo metabolic fluxes, hyperglycemia was associated with enhanced activation of hepatic glycogen synthase, decreased glycogen phosphorylase *a* activity, and enhanced hepatic glycogen content compared to the euglycemic controls (Table IV).

Thus, an acute elevation in the plasma glucose concentration inhibits HGP mainly through the stimulation of hepatic glycogen synthase and the inhibition of hepatic glycogen phosphorylase, while the activities of both glucokinase and G6Pase are not affected. However, a marked substrate-mediated increase in glucokinase flux (as reflected by the enhanced contribution of plasma-derived glucose to total G6Pase flux) allows the inhibition of HGP in the absence of any change in G6Pase flux and may contribute to the inhibition of HGP by maintaining the G6P concentration near the basal level despite the complete inhibition of glycogen breakdown (Fig. 1). This may facilitate the effects on the activity of hepatic glycogen enzymes, and perhaps more importantly, may contribute to maintain carbon flow through hepatic glycolysis and prevent an increase in gluconeogenic flux. Although such a scenario is speculative, it is in keeping with recent experimental evidence supporting

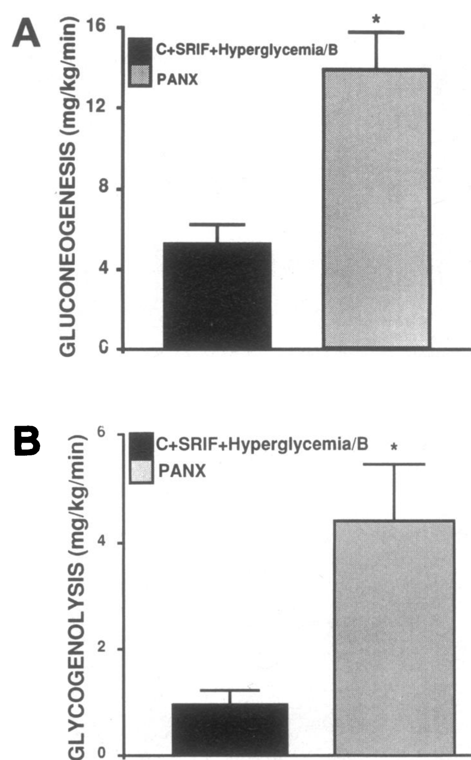

Figure 5. Rates of (*A*) gluconeogenesis and (*B*) hepatic glycogenolysis in control and 90% pancreatectomized diabetic rats, in the presence of similar conditions of hyperglycemia and moderate hypoinsulinemia (control rats received SRIF without insulin replacement; protocol 3B). $^*P < 0.01$ vs. C + SRIF + hyperglycemia/B.

the presence of an homeostatic regulation of hepatic glucose fluxes at the level of the G6P pool (7–11).

In control animals, the changes in HGP induced by changes in plasma glucose concentration correlated positively with the rate of glycogenolysis and negatively with the GC (Fig. 6). These correlations appear to further support the notion that in normal rats the effect of hyperglycemia on HGP is mainly mediated through its effects on hepatic glycogen breakdown and on glucose phosphorylation.

Since HGP is the main cause of postabsorptive hyperglycemia in diabetes, it is difficult to dissociate the enhanced HGP and hyperglycemia in the diabetic state. Thus, in the attempt to delineate the mechanism(s) responsible for enhanced HGP in diabetes, comparisons have been made between normoglycemic controls and hyperglycemic diabetic subjects (3–6). Due to the specific effects of hyperglycemia per se on hepatic glucose fluxes, the relative contribution of various metabolic pathways to the enhanced HGP in diabetes may be influenced by either chronic alterations in hepatic glucose metabolism or acute metabolic conditions at the time of the experimental study.

Similar to what has been previously reported in human NIDDM (3, 5), diabetic rats, in the present study, were characterized by a marked increase in HGP (~ 2-fold), glucose cycling (2.7-fold), and gluconeogenesis (2.7-fold), while the rate of hepatic glycogenolysis was similar to that in control animals (group 4 vs. 1). Furthermore, the increament in HGP, above control levels, could be accounted for entirely by the marked increase in gluconeogenic flux.

However, when diabetic and control rats were compared under similar conditions of hyperglycemia and moderate hypoinsulinemia, the relative contribution of the pathways of hepatic glucose metabolism to the marked increase in HGP were dramatically different. Although, in the diabetic group, HGP (3-fold) and TGO (1.7-fold) were markedly increased above control levels, the rate of GC was decreased by 21% (Fig. 4). Furthermore, both increased gluconeogenesis (+8.7 mg/kg · min) and hepatic glycogenolysis (+4.0 mg/kg · min) contributed to the increased HGP. The severe impairment in hepatic glucokinase and glycogen synthase activity and the about twofold increase in G6Pase activity seem to support the multifactorial origin and complexity of the alterations in hepatic glucose homeostasis which sustain the striking increase in postabsorptive HGP and fasting hyperglycemia.

Finally, the hepatic concentration of G6P may help to further speculate on the rate-determining step(s) responsible for the enhanced hepatic glucose output in this diabetic rat model. In fact, if the rate of formation of G6P exceeded the rate of its dephosphorylation one should be able to demonstrate an increase in the-steady-state level of G6P in the liver of diabetic

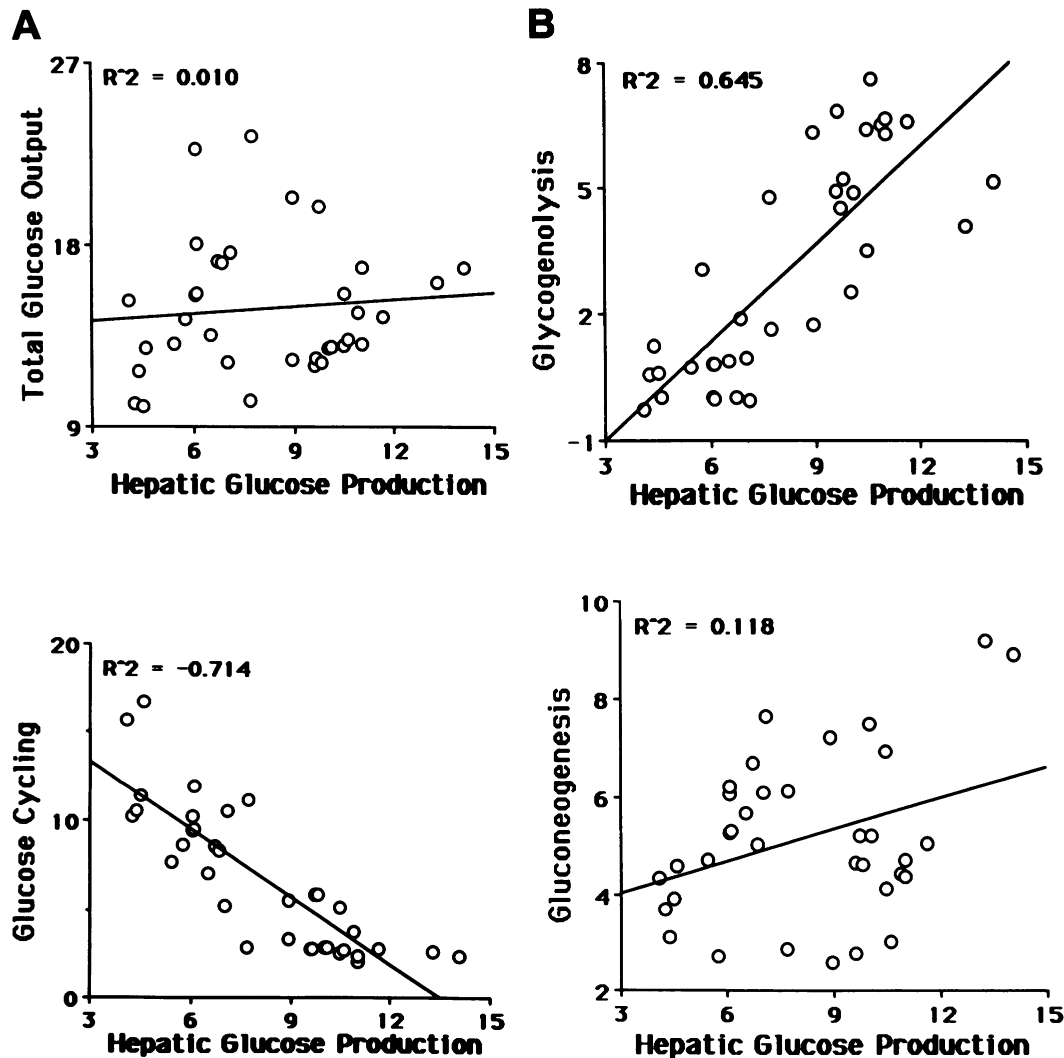

Figure 6. (*A*) Correlation between the rates of HGP and TGO (*upper panel*) and glucose/glycogen cycling (*lower panel*) in control rats. (*B*) Correlation between the rates of HGP and glycogenolysis (*upper panel*) and gluconeogenesis (*lower panel*) in control rats.

rats. On the contrary, if the rate of net dephosphorylation of hepatic G6P to glucose exceeded the rate of its formation (from gluconeogenesis and glycogenolysis), the steady-state G6P concentration should be lower in diabetic than in control liver. Since the hepatic G6P concentration was consistently lower in diabetic vs. control animals, this may suggest that the enhanced rate of dephosphorylation of G6P, rather than the increased rates of its formation, represented the rate-determining step for the increased HGP in the diabetic group. Furthermore, the marked differences observed in the correlates of HGP in the diabetic compared with the control groups suggest that increased flux through G6Pase is the major determinant of the increased HGP in this group and that the acute regulation of HGP by glucose is impaired after chronic hypoinsulinemia and hyperglycemia. The investigation of the latter issue will require further studies in which hepatic glucose fluxes in diabetic animals are examined in the presence of either normoglycemia or hyperglycemia.

In conclusion, our results indicate that (*a*) hyperglycemia, independent of changes in insulin concentration, causes a marked inhibition of HGP through the suppression of net glycogenolysis and through the increase in glucokinase flux, while the fluxes through gluconeogenesis and G6Pase are not significantly affected; and (*b*) under similar conditions of hyperglycemia and hypoinsulinemia, increased glycogenolysis and gluconeogenesis and decreased GC all contribute to the enhanced HGP in diabetic animals.

Acknowledgments

The authors thank Dr. Harry Shamoon (Albert Einstein College of Medicine) for critical reading of the manuscript and Drs. S. Pilkis and A. Lang (Stony Brook Medical School) for their assistance in setting up the glucokinase and glucose-6-phosphatase assays. We also thank Donna Banduch for her excellent technical assistance.

This work was supported by grants from the National Institutes of Health (R029-DK 42177), the Juvenile Diabetes Foundation (No. 1911127), and the American Diabetes Association and by the Albert Einstein Diabetes Research and Training Center (DK 20541).

References

1. Bell, P. M., R. G. Firth, and R. A. Rizza. 1986. Assessment of insulin action in insulin-dependent diabetes mellitus using [6 ^{14}C]glucose, [3 ^{3}H]glucose, and [2 ^{3}H]glucose. *J. Clin. Invest.* 78:1479–86.

2. DeFronzo, R. A. The triumvirate. 1988. B-cell, muscle, liver: a collusion responsible for NIDDM. *Diabetes.* 37:667–687.

3. Magnusson, I., D. L. Rothman, L. D. Katz, R. G. Shulman, and G. I. Shulman. 1992. Increased rate of gluconeogenesis in type II diabetes mellitus: a ^{13}C nuclear magnetic resonance study. *J. Clin. Invest.* 90:1323–1327.

4. Consoli, A., N. Nurjhan, J. J. Reilley, D. M. Bier, and J. E. Gerich. 1990. Mechanism of increased gluconeogenesis in non-insulin-dependent diabetes mellitus. *J. Clin. Invest.* 86:2038–2045.

5. Consoli, A., N. Nurjhan, F. Capani, and J. E. Gerich. 1989. Predominant role of gluconeogenesis in increased hepatic glucose production in NIDDM. *Diabetes.* 38:550–557.

6. Zawadzki, J. K., R. R. Wolfe, D. M. Mott, S. Lillioja, B. V. Howard, and C. Bogardus. 1988. Increased rate of Cori cycle in obese subjects with NIDDM and effect of weight reduction. *Diabetes.* 37:154–159.

7. Diamond, M. P., R. C. Rollins, K. E. Steiner, P. E. Williams, W. W. Lacy, and A. D. Cherrington. 1988. Effect of alanine concentration independent of changes in insulin and glucagon on alanine and glucose homeostasis in the conscious dog. *Metab. Clin. Exp.* 37:28–33.

8. Jennsen, T., N. Nurjhan, A. Consoli, and J. E. Gerich. 1990. Failure of substrate-induced gluconeogenesis to increase overall glucose appearance in normal humans. *J. Clin. Invest.* 86:489–497.

9. Jahoor, F., E. J. Peters, and R. R. Wolfe. 1990. The relationship between gluconeogenic substrate supply and glucose production in humans. *Am. J. Physiol.* 258:E288–E296.

10. Wolfe, R. B., F. Jahoor, and J. H. F. Shaw. 1986. Effect of alanine infusion on glucose and urea production in man. *J. Parenter. Enteral Nutr.* 11:109–111.

11. Puhakainen, I., V. A. Koivisto, and H. Yki-Jarvinen. 1991. No reduction in total hepatic glucose output by inhibition of gluconeogenesis with ethanol in NIDDM patients. *Diabetes.* 40:1319–27.

12. Soskin, S., and R. Levine. 1946. Carbohydrate Metabolism. University of Chicago Press, Chicago. 247–263.

13. Glinsman, W. H., E. P. Hern, and A. Lynch. 1969. Intrinsic regulation of glucose output by rat liver. *Am. J. Physiol.* 216:698–703.

14. Sacca, L., P. E. Hendler, and R. S. Sherwin. 1979. Hyperglycemia inhibits glucose production in man independent of changes in glucoregulatory hormones. *J. Clin. Endocrinol. Metabol.* 47:1160–1163.

15. Shulman, G. I., J. E. Lijenquist, P. E. Williams, W. W. Lacy, and A. D. Cherrington. 1978. Glucose disposal during insulinopenia somatostatin-treated dogs: the roles of glucose and glucagon. *J. Clin. Invest.* 62:487–491.

16. Shulman, G. I., W. W. Lacy, J. E. Lijenquist, U. Keller, P. E. Williams, and A. D. Cherrington. 1980. Effect of glucose, independent of changes in insulin and glucagon secretion, on alanine metabolism in the conscious dog. *J. Clin. Invest.* 65:496–505.

17. Bell, P. M., R. G. Firth, and R. A. Rizza. 1986. Effects of Hyperglycemia on glucose production and utilization in humans: measurements with [2 ^{3}H]-, [3 ^{3}H]-, and [6 ^{14}C]-glucose. *Diabetes.* 35:642–648.

18. Ader, M., G. Pacini, Y. J. Ysug, and R. N. Bergman. 1985. Importance of glucose per se to intravenous glucose tolerance: comparison of the minimal model prediction with direct measurements. *Diabetes.* 34:1092–1103.

19. Karlander, S., A. Roovete, M. Vranic, and S. Efendic. 1986. Glucose and fructose-6-phosphate cycle in humans. *Am. J. Physiol.* 251:E530–E536.

20. Efendic, S., S. Karlander, and M. Vranic. 1988. Mild type II diabetes markedly increases glucose cycling in the post-absorptive state and during glucose infusion irrespective of obesity. *J. Clin. Invest.* 81:1953–61.

21. Miyoshi, H., G. I. Shulman, E. J. Peters, M. H. Wolfe, D. Elahi, and R. R. Wolfe. 1988. Hormonal control of substrate cycling in humans. *J. Clin. Invest.* 81:1545–55.

22. Wajngot, A., A. Kahn, A. Giacca, M. Vranic, and S. Efendic. 1990. Dexamethasone increases glucose cycling, but not glucose production, in healthy subjects. *Am. J. Physiol.* 259:E626–E632.

23. Giaccari, A., and L. Rossetti. 1992. Predominant role of gluconeogenesis in the hepatic glycogen repletion of diabetic rats. *J. Clin. Invest.* 89:36–45.

24. Granner, D. K., and T. L. Andreone. 1985. Insulin modulation of gene expression. *Diabetes Metab. Rev.* 1:139–170.

25. Foglia, V. G. 1944. Caracterhsticas de la diabetes en la rata. *Rev. Soc. Argent. Biol.* 20:21–37.

26. Bonner-Weir, S., D. F. Trent, and G. C. Weir. 1983. Partial pancreatectomy in the rat and subsequent defect in glucose-induced insulin release. *J. Clin. Invest.* 71:1544–1553.

27. Rossetti, L., and A. Giaccari. 1990. Relative contribution of glycogen synthesis and glycolysis to insulin-mediated glucose uptake: a dose-response euglycemic clamp study in normal and diabetic rats. *J. Clin. Invest.* 85:1785–1792.

28. Rossetti, L., D. Smith, G. I. Shulman, D. Papachristou, and R. A. DeFronzo. 1987. Correction of hyperglycemia with phlorizin normalizes tissue sensitivity to insulin in diabetic rats. *J. Clin. Invest.* 79:1510–1515.

29. Rossetti, L., and M. R. Laughlin. 1989. Correction of chronic hyperglycemia with vanadate, but not phlorizin, normalizes in vivo glycogen repletion and in vitro glycogen synthase activity in diabetic skeletal muscle. *J. Clin. Invest.* 84:892–899.

30. Farrace, S., and L. Rossetti. 1992. Hyperglycemia markedly enhances skeletal muscle glycogen synthase activity in diabetic, but not in normal conscious rats. *Diabetes.* 41:1453–1463.

31. Thomas, J. A., K. K. Schlender, and J. Larner. 1968. A rapid filter paper assay for UDPG-glycogen glucosyltransferase, including an improved biosynthesis of UDP [^{14}C]glucose. *Anal. Biochem.* 25:486–499.

32. Rossetti, L., S. Farrace, S. B. Choi, A. Giaccari, L. Sloan, S. Frontoni, and M. S. Katz. 1993. Multiple metabolic effects of calcitonin gene-related peptide (CGRP) in conscious rats: relationship to its regulation of glycogen synthase and adenylate cyclase. *Am. J. Physiol.* 264:E1–E10.

33. Davidson, A. L., and W. J. Arion. 1987. Factors underlying significant underestimations of glucokinase activity in crude liver extracts: physiological implications of higher cellular activity. *Arch. Biochem. Biophys.* 253:156–167.

34. Burchell, A., R. Hume, and B. Burchell. 1988. A new microtechnique for the analysis of the human hepatic microsomal glucose-6-phosphatase system. *Clin. Chim. Acta.* 173:183–192.

35. Michal, G. 1985. Methods of Enzymatic Analysis. Volume VI. N.U. Bergmeyer, editor. VCH Verlagsgesellschaft mbH, Weinheim, FRG. 191–198.

36. Giaccari, A., and L. Rossetti. 1989. Isocratic high-performance liquid chromatographic determination of the concentration and specific radioactivity of phosphoenolpyruvate and uridine diphosphate glucose in tissue extracts. *J. Chromatogr.* 497:69–78.