

# Fructose Improves the Ability of Hyperglycemia Per Se to Regulate Glucose Production in Type 2 Diabetes

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The ability of hyperglycemia per se to suppress endogenous glucose production (GP) is blunted in type 2 diabetes. This could be due in part to decreased glucose-induced flux through glucokinase (GK). Because fructose activates hepatic GK, we examined whether catalytic amounts of fructose could restore inhibition of GP by hyperglycemia in humans with type 2 diabetes. Glucose fluxes ( $[3\text{-}^3\text{H}]\text{glucose}$ ) were measured during euglycemia (5 mmol/l) and after abrupt onset of hyperglycemia (10 mmol/l; variable dextrose infusion) under fixed hormonal conditions (somatostatin infusion for 6 h with basal insulin/glucagon/growth hormone replacement). A total of 10 subjects with moderately controlled type 2 diabetes and 7 age- and BMI-matched nondiabetic subjects were studied on up to three separate occasions under the following conditions: without fructose ( $F^-$ ) or with infusion of fructose at two dosages: 0.6 mg/kg · min (low F) and 1.8 mg/kg · min (high F). Although GP failed to decrease in response to hyperglycemia in type 2 diabetes, the coinfusion of both doses of fructose was associated with comparable decreases in GP in response to hyperglycemia (low F = -27%, high F = -33%;  $P < 0.01$  vs.  $F^-$  at both dosages), which approached the 44% decline in GP observed without fructose in the nondiabetic subjects. GP responses to hyperglycemia were not altered by the addition of fructose in the nondiabetic group (low F = -47%, high F = -42%;  $P > 0.05$  vs.  $F^-$ ). Thus, the administration of small amounts of fructose to type 2 diabetic subjects partially corrected the regulation of GP by hyperglycemia per se, yet did not affect this regulation in the nondiabetic subjects. This suggests that the liver's inability to respond to hyperglycemia in type 2 diabetes, likely caused by impaired GK activity, contributes substantially to the increased GP in these individuals. *Diabetes* 51:606–614, 2002

An excessive rate of endogenous glucose production (GP) is the major contributor to fasting hyperglycemia in type 1 and type 2 diabetes (1–3). In nondiabetic animals and humans, a rapid and direct regulation of hepatic glucose fluxes by glucose per se helps maintain plasma glucose levels within a narrow range (4,5). Thus, in response to acute increases in plasma glucose concentration, GP is inhibited independently of hormonal signals. However, type 2 diabetes is associated with increased GP, despite elevated plasma concentrations of both glucose and insulin (6), and this inappropriate increase in GP contributes to the vicious cycle of worsening hyperglycemia.

The normal inhibitory effect of hyperglycemia on GP requires an increased flux of glucose through glucokinase (GK), which is the principal catalyst for glucose phosphorylation in hepatocytes and pancreatic  $\beta$ -cells (7). GK displays a sigmoidal saturation curve for glucose, with a predominance of a low-affinity form of the enzyme within the physiological range of plasma glucose levels (8), making this enzyme highly sensitive to changes in plasma glucose levels. A hepatic “autoregulatory” mechanism normally operates at the level of the glucose-6-phosphate (G-6-P) pool in response to increased phosphorylation of plasma glucose to G-6-P, resulting in suppression of GP (9,10). The rate of hepatic glucose phosphorylation depends largely on the mass effect of glucose, i.e., its portal concentration, and on the in vivo activity of GK (11,12). Thus, the diminished ability of hyperglycemia per se to inhibit GP in type 2 diabetes may reflect suboptimal increases in hepatic glucose phosphorylation in response to hyperglycemia, such that several complex molecular and biochemical responses would not be appropriately affected by changes in portal glucose levels (13,14). Indeed, animal models with decreased hepatic GK activity manifest an inability to regulate GP in response to hyperglycemia (15,16).

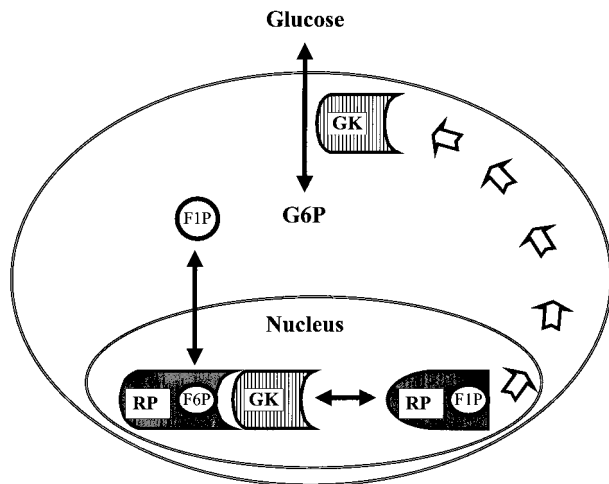
Furthermore, some recent evidence suggests that a defect in hepatic glucose phosphorylation might underlie the lack of suppression of GP by hyperglycemia in human diabetes (17–19). In the case of maturity-onset diabetes of the young (MODY)-2 (17), an inherited defect in GK enzymatic activity contributes to the pathophysiology of hyperglycemia. Alternatively, defects in GK activity can be acquired secondarily to associated hormonal and metabolic alterations in type 2 diabetes (18,19). Furthermore, some of the alterations in glucose fluxes observed in

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$F^-$ , without fructose; FFA, free fatty acid; G-6-P, glucose-6-phosphate; G-6-Pase, glucose-6-phosphatase; GK, glucokinase; GP, glucose production; GCRC, General Clinical Research Center; high F, with infusion of fructose at 1.8 mg/kg · min; low F, with infusion of fructose at 0.6 mg/kg · min; MODY, maturity-onset diabetes of the young; RP, regulatory protein.



**FIG. 1.** Schematic representation of the regulation of GK translocation in liver. GK is held in the nucleus by high-affinity binding to an RP, such that its configuration and location are not amenable to phosphorylating incoming glucose. Increased availability of fructose results in generation of fructose-1-P (F1P) via fructokinase. Fructose-1-P displaces fructose-6-P (F6P) from its binding site on the RP. The binding of fructose-1-P to the RP alters the configuration of the RP, such that it no longer binds GK. The liberated and activated GK immediately translocates to the cytoplasm, where it is in an appropriate position to phosphorylate incoming glucose.

subjects closely resemble those observed in rodent models of impaired hepatic GK activity (15,16).

Short-term metabolic regulation of GK activity is provided by a 68-kDa regulatory protein (RP) that binds GK with high affinity (20). Indeed, hepatic GK is mainly located in the nucleus at low glucose concentrations, in tight association with its inhibitory RP (21–25). Fructose-1-phosphate, generated from fructose through the action of hepatic fructokinase, binds to the RP and thereby decreases its affinity for GK (20–23). This permits the immediate translocation of GK to the cytosol, where it can readily phosphorylate incoming glucose (Fig 1.) (26,27). Furthermore, the resulting conformational change in GK increases its affinity for glucose (28).

The operation of this rapid regulatory mechanism has recently been demonstrated in conscious dogs by administering small amounts of fructose either intraportally or intraduodenally (29,30). These relatively low concentrations of fructose (150  $\mu\text{mol/l}$ ) markedly improved glucose tolerance during the infusion of glucose at a fixed rate. Because the hepatic glycogen synthase enzyme is known to be activated by GK (31,32), further *in vivo* support for the ability of fructose to activate GK was provided by the recent observation that small amounts of fructose exert stimulatory effects on glycogen synthesis in humans (33).

We hypothesized that impaired GK activity is at least partly responsible for the lack of glucose-mediated modulation of hepatic glucose fluxes in human type 2 diabetes. To test this hypothesis, we used fructose infusions to transiently “rescue” *in vivo* GK activity in patients with type 2 diabetes under “pancreatic clamp” conditions. Our results indicate that these relatively low rates of fructose infusion can significantly affect the response of hepatic glucose fluxes to hyperglycemia in type 2 diabetic subjects.

## RESEARCH DESIGN AND METHODS

A total of 10 subjects with type 2 diabetes (1 woman and 9 men) and 7 age- and weight-matched nondiabetic control subjects (2 women and 5 men) were studied on at least two separate occasions. Subjects with type 2 diabetes had an HbA<sub>1c</sub> value (mean  $\pm$  SE) of  $8.1 \pm 0.4\%$ , an average age of  $54.6 \pm 3.2$  years, and a mean BMI of  $28.5 \pm 1.5$  kg/m<sup>2</sup>. The average duration of type 2 diabetes was  $11.3 \pm 2.4$  years. Five subjects were treated with oral antihyperglycemic agents alone (two with metformin and troglitazone, one with metformin and glimepiride, one with metformin and glipizide, and one with metformin alone), three with a combination of insulin and oral agents, and two with insulin alone. Except for diabetes, all were in general good health. The nondiabetic healthy volunteers had an average BMI of  $29.9 \pm 1.4$  kg/m<sup>2</sup>, an average age of  $51.8 \pm 5.1$  years, and a mean HbA<sub>1c</sub> of  $5.2 \pm 0.3\%$ . None of the nondiabetic subjects were taking any medication, and none had a first-degree relative with type 2 diabetes. Eligibility for the study was determined by history, physical examination, and hematologic and biochemical tests. Individuals with anemia, bleeding disorders, or recent weight changes were excluded. Informed written consent was obtained in accordance with the policy of the Committee on Clinical Investigations of the Albert Einstein College of Medicine.

**Experimental design.** Type 2 diabetic subjects discontinued their antihyperglycemic medications before the study according to the following guidelines: sulfonylureas and metformin were discontinued at least 48 h before the study, troglitazone was withheld for 8 days, and long-acting insulin was withheld the evening before the study. Type 2 diabetic subjects were admitted to the General Clinical Research Center (GCRC) the evening before the study for low-dose overnight insulinization in order to gradually lower plasma glucose levels to  $<140$  mg/dl by morning. Beginning at 10:00 P.M. on the night before the study, patients were asked to fast, and a variable intravenous infusion of insulin was started. The insulin infusion rate overnight was adjusted according to an algorithm based on hourly blood glucose measurements, assuring a gradual normalization of plasma glucose levels (34). The experimental protocol was initiated the following morning. Nondiabetic subjects were admitted to the GCRC on the morning of the study, after a comparable overnight fast.

An 18-gauge catheter was inserted in an antecubital vein for infusions, and a contralateral hand vein was cannulated in a retrograde fashion for arterialized venous blood sampling. To obtain arterialized venous blood, this hand was kept in a warming plexiglass box maintained at 55°C. The experimental protocols lasted 6 h, and all consisted of an initial 4-h euglycemic period followed by a 2-h hyperglycemic period. At  $t = 0$  min, a primed-continuous infusion of high-performance liquid chromatography-purified [<sup>3</sup>-<sup>3</sup>H]glucose (New England Nuclear, Boston, MA) was started (bolus 22  $\mu\text{Ci}$ ; continuous infusion rate 0.15  $\mu\text{Ci/min}$ ) via an indwelling catheter.

At  $t = 0$  min, an infusion containing somatostatin (250  $\mu\text{g/h}$ ; Bachem, King of Prussia, PA), growth hormone ( $3.0$  ng  $\cdot$  kg<sup>-1</sup>  $\cdot$  min<sup>-1</sup>; Genentech, San Francisco, CA), glucagon ( $1.0$  ng  $\cdot$  kg<sup>-1</sup>  $\cdot$  min<sup>-1</sup>; Eli Lilly, Indianapolis, IN), and albumin (1 mg/ml, diluted in saline) was initiated and maintained throughout the study. At  $t = 0$  min, an infusion of insulin (Novolin Regular, prepared in albumin-containing saline; Novo-Nordisk, Princeton, NJ) was also initiated. Plasma glucose concentrations were initially measured at 15-min intervals (for the period  $t = 0$ –240 min), and the rate of insulin infusion was adjusted to maintain euglycemia (90–100 mg/dl), without the need for exogenous glucose infusion. When a stable rate of insulin infusion was determined, it was kept constant for the remainder of the study. This occurred within the first 150 min of the euglycemic period ( $t = 0$ –150 min).

During the remaining 90 min of the euglycemic period, the “individualized” basal rate of insulin infusion was kept constant, and ambient euglycemia prevailed until  $t = 240$  min. At  $t = 240$  min, plasma glucose levels were rapidly increased by 90 mg/dl and clamped at 180 mg/dl for the following 2 h (until  $t = 360$  min), using an exogenous infusion of 20% glucose. Tracer ([<sup>3</sup>-<sup>3</sup>H]glucose) was added to the “cold” 20% glucose ( $\sim 0.1$   $\mu\text{Ci/ml}$ ) to maintain constant glucose specific activity during hyperglycemia (35). All other infusions were maintained at the rates established during the euglycemic period.

To determine the effect of GK on the ability of hyperglycemia per se to regulate GP, each subject was studied under the euglycemic-hyperglycemic pancreatic clamp conditions described above, with and without the addition of fructose. At 15 min before the initiation of hyperglycemia ( $t = 225$  min), infusions of saline (without fructose [F<sup>-</sup>]) or fructose at two dosages (0.6 mg/kg  $\cdot$  min [low F] and 1.8 mg/kg  $\cdot$  min [high F]) were initiated. These infusion rates of fructose were selected in order to achieve plasma fructose levels of  $\sim 100$  and 300  $\mu\text{mol/l}$ , respectively (29,36–38). Such low circulating levels of fructose would be regulatory for GK translocation, without providing increased substrate for gluconeogenesis (39). Thus, each subject was studied on at least two separate occasions under the following experimental conditions: 1) hyperglycemia (180 mg/dl) and 2) hyperglycemia with fructose at two dosages, such that every subject underwent a baseline (F<sup>-</sup>) study and at least one fructose infusion study (low F and/or high F). Of the total of 41 clamp

studies performed, five nondiabetic subjects and two type 2 diabetic subjects were studied at both fructose dosages, whereas one nondiabetic subject and four type 2 diabetic subjects received only the low dose, and one nondiabetic subject and four type 2 diabetic subjects received only the high dose. Of note, metabolic control in the type 2 diabetic subjects was very stable, such that there was no more than a 0.2% difference in HbA<sub>1c</sub> in any individual on separate study days, which were separated in time by several months.

Blood was sampled at 15-min intervals for measurement of plasma glucose and specific activity of [3-<sup>3</sup>H]glucose, respectively, and at 30-min intervals for plasma insulin, C-peptide, glucagon, fructose, free fatty acids (FFAs), lactate, and glycerol. During the hyperglycemic study intervals, blood was sampled at 5-min intervals for measurement of plasma glucose to provide immediate titration of rates of 20% glucose infusion. The tracer, glucose, and hormone infusions were terminated upon completion of the study. Insulin infusion was continued in the type 2 diabetic patients for an additional 15 min. Subjects received a meal and were discharged, and type 2 diabetic individuals immediately resumed their usual therapeutic regimens.

**Analytical procedures.** Plasma glucose was measured with a Beckman glucose analyzer (Fullerton, CA) by use of the glucose oxidase method. Plasma [3-<sup>3</sup>H]glucose radioactivity was measured in duplicate on the supernatants of barium hydroxide–zinc sulfate precipitates (Somogyi procedure) of plasma samples after evaporation to dryness to eliminate tritiated water. Plasma-tritiated water specific activity was determined by liquid scintillation-counting of the protein-free supernatant (Somogyi filtrate) before and after evaporation to dryness (40).

Plasma insulin, C-peptide, and glucagon were determined by radioimmunoassay (41). Plasma FFAs and glycerol were measured using colorimetric enzymatic methods (34), and plasma lactate was determined by an enzymatic spectrophotometric assay (42). HbA<sub>1c</sub> was measured by ion-exchange chromatography, with an upper normal limit of 6.2% (43). After deproteinization of whole blood samples, plasma fructose levels were measured by the method of Beutler (44).

**Calculations.** Rates of glucose appearance ( $R_a$ ) and glucose disappearance ( $R_d$ , or glucose uptake) were calculated using Steele's steady-state equation (45). Rates of endogenous GP were determined by subtracting rates of glucose infusion from the tracer-determined  $R_a$ . Data for glucose turnover, plasma hormones, and substrate concentrations represent the mean values during the final 60 min of the euglycemic period ( $t = 180$ – $240$  min) and the final 60 min of the hyperglycemic period ( $t = 300$ – $360$  min).

Statistical analysis of the data over time was performed using Proc Mixed in SAS System version 6.12 (SAS Institute, Cary, NC). For comparisons between two test conditions, Student's  $t$  test was used, with paired  $t$  tests for comparisons of euglycemic and hyperglycemic intervals and nonpaired  $t$  tests to compare the two subject groups. For comparisons between the three treatment categories ( $F^-$ , low F, and high F), repeated-measures ANOVA was applied.

## RESULTS

Given the paired nature of the studies, most comparisons will be made between the fructose studies at both dosages and the baseline studies for the same group of subjects (i.e., low F and high F vs. their respective  $F^-$  studies). In some cases, overall comparisons will be made between type 2 diabetic and nondiabetic subjects under baseline conditions by combining all  $F^-$  study data, as outlined in the text below.

**Plasma glucose levels and insulin infusion rates.** On the morning of the study, plasma glucose levels averaged  $121 \pm 11$  mg/dl in the type 2 diabetic subjects and  $103 \pm 4$  mg/dl in the nondiabetic subjects. Plasma glucose levels were similar in type 2 diabetic and nondiabetic subjects during both the euglycemic study period ( $t = 180$ – $240$  min:  $91.7 \pm 2.2$  mg/dl in type 2 diabetic subjects and  $90.9 \pm 2.9$  mg/dl in nondiabetic subjects) and the hyperglycemic study period ( $t = 300$ – $360$  min:  $181.4 \pm 1.2$  mg/dl in type 2 diabetic subjects and  $180.5 \pm 1.3$  mg/dl in nondiabetic subjects). Importantly, the amplitude of the change in the ambient glucose concentrations generated with the hyperglycemic clamp was comparable in both groups.

The rate of overnight insulin infusion required to maintain plasma glucose in the target range of 90–120 mg/dl in

the type 2 diabetic group averaged  $2.8 \pm 0.6$  units/h. The average insulin infusion rate needed to maintain euglycemia during the final 30 min of the insulin adjustment period and thereafter was similar in both groups ( $0.25 \pm 0.03$  mU  $\cdot$  kg<sup>-1</sup>  $\cdot$  min<sup>-1</sup> in type 2 diabetic subjects and  $0.26 \pm 0.05$  mU  $\cdot$  kg<sup>-1</sup>  $\cdot$  min<sup>-1</sup> nondiabetic subjects,  $P > 0.05$ ). Paired analyses revealed that insulin infusion rates did not differ between the fructose studies (at both dosages) and their respective  $F^-$  studies. Of note, the overnight insulin infusions used in the type 2 diabetic group were comparable to those used by Wise et al. (46), who noted that hepatic (but not peripheral) insulin action was completely restored. Because our low infusion rates of insulin during the clamp studies were only those required to maintain euglycemia without infusion of exogenous glucose, hepatic insulin action had presumably recovered completely and was therefore not different from the nondiabetic subjects. It is important to note, however, that such infusion rates of insulin overnight do not correct hepatic or peripheral glucose effectiveness (47).

**Plasma hormone levels.** After an overnight fast in all subjects and insulin infusion in the type 2 diabetic subjects, plasma insulin concentrations were higher in type 2 diabetic subjects (overall fasting levels  $31.0 \pm 3.3$   $\mu$ U/ml) than in nondiabetic subjects ( $11.6 \pm 1.1$   $\mu$ U/ml,  $P < 0.01$ ) and were similar on separate study days within both groups of subjects (Table 1). Furthermore, the plasma insulin levels did not differ between the euglycemic and hyperglycemic study periods in any group or study type ( $P > 0.05$  for euglycemic versus hyperglycemic study periods). C-peptide levels were comparable in both groups at the onset of the studies ( $0.58 \pm 0.05$  pmol/ml in the nondiabetic subjects and  $0.47 \pm 0.16$  pmol/ml in the type 2 diabetic subjects,  $P > 0.05$ ) and were suppressed by somatostatin infusion in all groups throughout the duration of the studies.

Plasma glucagon levels remained stable throughout the euglycemic and hyperglycemic study periods in all groups.

**Plasma substrate levels.** Plasma fructose levels were nondetectable ( $<5$   $\mu$ mol/l) in all subjects throughout the baseline ( $F^-$ ) studies and during the initial 225 min of the low and high F studies, before the initiation of fructose infusion. During the fructose infusion in the low F studies, plasma fructose levels averaged  $131 \pm 15$   $\mu$ mol/l from  $t = 270$ – $300$  min and  $121 \pm 20$   $\mu$ mol/l from  $t = 330$ – $360$  min in the nondiabetic subjects, and they averaged  $114 \pm 14$   $\mu$ mol/l from  $t = 270$ – $300$  min and  $121 \pm 9$   $\mu$ mol/l from  $t = 330$ – $360$  min in the type 2 diabetic subjects. During the comparable time periods in the high F fructose infusion studies, plasma fructose levels averaged  $287 \pm 16$  and  $276 \pm 13$   $\mu$ mol/l, respectively, in the nondiabetic subjects and  $292 \pm 18$  and  $267 \pm 16$   $\mu$ mol/l in the type 2 diabetic subjects ( $P < 0.05$  vs. low F at all time points).

Plasma FFA levels were comparable in all subjects at the beginning of the studies ( $t = 0$ ), averaging  $557 \pm 66$   $\mu$ mol/l overall in the nondiabetic subjects and  $421 \pm 71$   $\mu$ mol/l in the type 2 diabetic subjects ( $P > 0.05$ ), reflecting the effects of overnight insulin infusion in the latter (Table 2). During most euglycemic and hyperglycemic study periods, the plasma FFA concentrations were significantly higher in the type 2 diabetic group than in the nondiabetic group.

TABLE 1

Plasma hormone concentrations during low-dose fructose (low F) infusion studies and the corresponding control (F<sup>-</sup>) studies in type 2 diabetic (*n* = 6) and nondiabetic (*n* = 6) subjects and during high-dose fructose (high F) infusion studies matched with control (F<sup>-</sup>) studies in type 2 diabetic (*n* = 6) and nondiabetic (*n* = 6) subjects.

	Insulin (μU/ml)		Glucagon (pg/ml)		C-peptide (pmol/ml)	
	Nondiabetic subjects	Type 2 diabetic subjects	Nondiabetic subjects	Type 2 diabetic subjects	Nondiabetic subjects	Type 2 diabetic subjects
Control (F <sup>-</sup> )						
Euglycemia ( <i>t</i> = 180–240 min)	23 ± 2	24 ± 5	86 ± 2	95 ± 9	0.05 ± 0.01	0.05 ± 0.01
Hyperglycemia ( <i>t</i> = 300–360 min)	24 ± 3	22 ± 4	82 ± 4	91 ± 9	0.08 ± 0.02	0.04 ± 0.01
Low-dose fructose						
Euglycemia ( <i>t</i> = 180–240 min)	22 ± 2	30 ± 7	84 ± 6	85 ± 11	0.07 ± 0.01	0.09 ± 0.04
Hyperglycemia ( <i>t</i> = 300–360 min)	22 ± 4	23 ± 5	84 ± 8	80 ± 9	0.08 ± 0.01	0.07 ± 0.04
Control (F <sup>-</sup> )						
Euglycemia ( <i>t</i> = 180–240 min)	23 ± 1	27 ± 6	92 ± 6	92 ± 6	0.06 ± 0.01	0.09 ± 0.03
Hyperglycemia ( <i>t</i> = 300–360 min)	24 ± 2	26 ± 6	88 ± 7	89 ± 6	0.09 ± 0.02	0.05 ± 0.01
High-dose fructose						
Euglycemia ( <i>t</i> = 180–240 min)	24 ± 3	35 ± 7	97 ± 6	90 ± 7	0.05 ± 0.01	0.06 ± 0.01
Hyperglycemia ( <i>t</i> = 300–360 min)	24 ± 3	32 ± 6	85 ± 7	82 ± 5	0.10 ± 0.02	0.05 ± 0.02

Data are means ± SE.

There was no significant change in plasma FFA concentrations in either group of subjects with the addition of fructose at either dosage. Although there was a trend toward higher glycerol levels during euglycemic and hyperglycemic clamp intervals in the type 2 diabetic subjects, the difference only reached significance during euglycemia in the control studies for the high F studies. Fructose infusion did not affect glycerol levels in any of the groups. It is important to note that the rates of peripheral insulin infusion required to maintain euglycemia in the nondiabetic subjects under the clamp conditions (including infusion of counterregulatory hormones) resulted in plasma insulin levels that were higher than their measured fasting levels. Over the course of the 6-h studies, these modest elevations in plasma insulin levels above habitual fasting levels (though still within the basal range) resulted in mild, gradual decreases in plasma FFA levels in the nondiabetic group. Also, it is possible that

glucose may have increased fatty acid re-esterification in the nondiabetic subjects, given that levels of FFA decreased with hyperglycemia, whereas plasma glycerol levels were unchanged.

For all study types, there was a trend toward lower plasma lactate levels in the type 2 diabetic subjects than in the nondiabetic subjects, although this was only significant for the F<sup>-</sup> studies during hyperglycemia. Plasma lactate levels rose in a dose-dependent manner with the infusion of fructose in both the nondiabetic and type 2 diabetic subjects. Indeed, increased activation of hepatic GK by fructose would be expected to provide more G6P (from incoming glucose) for glycolysis, thereby raising plasma lactate levels. Thus, the suppression of GP during hyperglycemia in the presence of fructose in the type 2 diabetic subjects was all the more noteworthy, considering the increase in potential substrate for gluconeogenesis.

TABLE 2

Plasma substrate concentrations during low-dose fructose (low F) infusion studies and the corresponding control (F<sup>-</sup>) studies in type 2 diabetic (*n* = 6) and nondiabetic (*n* = 6) subjects and during high-dose fructose (high F) infusion studies matched with control (F<sup>-</sup>) studies in type 2 diabetic (*n* = 6) and nondiabetic (*n* = 6) subjects.

	FFA (μmol/l)		Glycerol (μmol/l)		Lactate (mmol/l)	
	Nondiabetic subjects	Type 2 diabetic subjects	Nondiabetic subjects	Type 2 diabetic subjects	Nondiabetic subjects	Type 2 diabetic subjects
Control (F <sup>-</sup> )						
Euglycemia ( <i>t</i> = 180–240 min)	186 ± 28	413 ± 109	57 ± 10	68 ± 12	0.83 ± 0.05	0.66 ± 0.10
Hyperglycemia ( <i>t</i> = 300–360 min)	139 ± 8*	398 ± 94	68 ± 13	73 ± 14	1.05 ± 0.13	0.71 ± 0.13
Low-dose fructose						
Euglycemia ( <i>t</i> = 180–240 min)	204 ± 32	393 ± 166	73 ± 7	76 ± 10	0.88 ± 0.11†	0.68 ± 0.08‡
Hyperglycemia ( <i>t</i> = 300–360 min)	153 ± 27	324 ± 94	85 ± 10	89 ± 18	1.25 ± 0.15	0.87 ± 0.11
Control (F <sup>-</sup> )						
Euglycemia ( <i>t</i> = 180–240 min)	227 ± 46*	361 ± 64	44 ± 4*	65 ± 6	0.83 ± 0.01	0.65 ± 0.14
Hyperglycemia ( <i>t</i> = 300–360 min)	150 ± 13§	404 ± 45	51 ± 8	73 ± 9	1.05 ± 0.18*	0.65 ± 0.12
High-dose fructose						
Euglycemia ( <i>t</i> = 180–240 min)	183 ± 19*	297 ± 42	44 ± 6	70 ± 14	0.98 ± 0.19‡	0.83 ± 0.14‡
Hyperglycemia ( <i>t</i> = 300–360 min)	148 ± 13§	332.5 ± 4	52 ± 4	81 ± 20	1.45 ± 0.24	1.24 ± 0.23

Data are means ± SE. \**P* < 0.05 for nondiabetic vs. type 2 diabetic subjects; †*P* < 0.05 for euglycemia vs. hyperglycemia; ‡*P* < 0.01 for euglycemia vs. hyperglycemia; §*P* < 0.01 for nondiabetic vs. type 2 diabetic subjects; ||*P* < 0.05 for low F vs. high F.

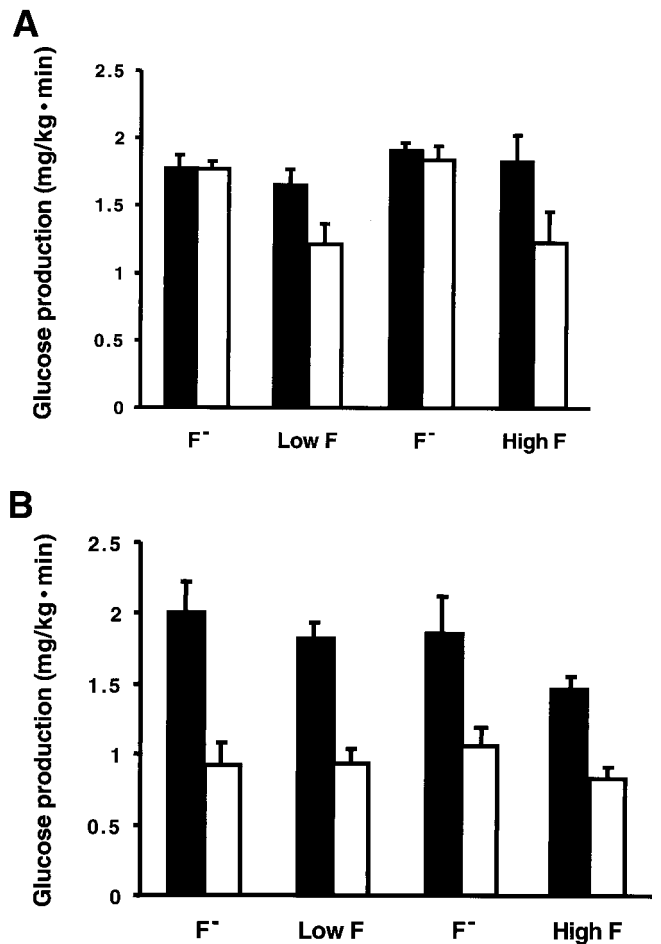
**Glucose infusion rate.** The average rate of infusion of glucose required to maintain the target hyperglycemic plateau during the last 60 min of the hyperglycemic period was decreased by >50% in type 2 diabetic subjects compared with nondiabetic subjects in the baseline studies ( $F^-$ :  $0.8 \pm 0.1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  in type 2 diabetic vs.  $2.7 \pm 0.4 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  in nondiabetic subjects,  $P < 0.05$  for type 2 diabetic vs. nondiabetic subjects). Furthermore, the glucose infusion rate increased with fructose in the type 2 diabetic subjects ( $1.3 \pm 0.2$  and  $1.7 \pm 0.3 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  for low and high F, respectively;  $P < 0.05$  vs.  $F^-$ ). However, fructose administration did not affect the glucose infusion rate in the nondiabetic subjects ( $2.3 \pm 0.2$  and  $2.3 \pm 0.3 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  for low and high F, respectively;  $P > 0.05$  vs.  $F^-$ ).

**Glucose production.** Glucose specific activity was constant after tracer equilibration during both euglycemia and hyperglycemia in each group. GP was similar in both groups during the euglycemic study periods in the  $F^-$  studies and during the fructose infusion studies ( $P > 0.05$  for nondiabetic vs. type 2 diabetic subjects). To determine the effect of chronic hyperglycemia on the effectiveness of glucose to suppress GP, we first compared the responses to hyperglycemia in all  $F^-$  studies between nondiabetic and type 2 diabetic subjects. During hyperglycemia in the  $F^-$  studies, GP was suppressed by ~45% in the nondiabetic subjects ( $1.0 \pm 0.1 \text{ mg/kg} \cdot \text{min}$  for hyperglycemia vs.  $1.9 \pm 0.2 \text{ mg/kg} \cdot \text{min}$  for euglycemia,  $P < 0.01$ ), whereas the type 2 diabetic subjects failed to show any suppression of GP with hyperglycemia ( $1.8 \pm 0.1$  vs.  $1.8 \pm 0.1 \text{ mg/kg} \cdot \text{min}$ ,  $P > 0.05$ ).

The coinfusion of fructose at the lower infusion rate was able to partially restore the normal response of GP to hyperglycemia in type 2 diabetes (low F:  $1.2 \pm 0.1 \text{ mg/kg} \cdot \text{min}$  for hyperglycemia vs.  $1.7 \pm 0.1 \text{ mg/kg} \cdot \text{min}$  for euglycemia,  $27 \pm 6\%$  suppression,  $P < 0.01$  vs.  $F^-$ ) relative to the  $F^-$  studies in the same subjects ( $F^-$ :  $1.8 \pm 0.1$  vs.  $1.8 \pm 0.1 \text{ mg/kg} \cdot \text{min}$ ,  $0 \pm 6\%$  suppression). The higher infusion rate of fructose restored the hyperglycemia-induced suppression of GP to a similar degree (high F:  $1.2 \pm 0.2$  vs.  $1.8 \pm 0.2 \text{ mg/kg} \cdot \text{min}$ ,  $33 \pm 8\%$  suppression,  $P < 0.05$  vs.  $3 \pm 6\%$  suppression in same subjects in  $F^-$  studies). Indeed, there was no difference in effect size between the dosages of fructose ( $P > 0.05$ ) (Fig. 2A).

In the nondiabetic subjects, the infusion of fructose did not alter the response of GP to hyperglycemia at either the low (low F:  $0.9 \pm 0.1 \text{ mg/kg} \cdot \text{min}$  for hyperglycemia vs.  $1.8 \pm 0.1 \text{ mg/kg} \cdot \text{min}$  for euglycemia,  $47 \pm 7\%$  suppression,  $P > 0.05$  for percent suppression vs.  $F^-$  in same subjects) or the high infusion rate (high F:  $0.8 \pm 0.1$  vs.  $1.5 \pm 0.1 \text{ mg/kg} \cdot \text{min}$ ,  $42 \pm 5\%$  suppression,  $P > 0.05$  for percent suppression vs.  $F^-$  in same subjects) (Fig. 2B).

**Glucose uptake.** The percent increase in glucose uptake between the euglycemic and hyperglycemic study periods was ~94% for nondiabetic subjects in the combined baseline studies ( $F^-$ :  $1.9 \pm 0.2 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  for euglycemia vs.  $3.7 \pm 0.1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  for hyperglycemia), whereas there was a more modest increase of ~50% overall in the type 2 diabetes subjects ( $F^-$ :  $1.8 \pm 0.1$  vs.  $2.6 \pm 0.1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ,  $P < 0.05$  for comparison of percent change in glucose uptake between type 2 diabetic and nondiabetic subjects). Thus, as previously reported (5), in addition to



**FIG. 2.** Endogenous GP during euglycemic-hyperglycemic studies in the type 2 diabetic and nondiabetic subjects, without ( $F^-$ ) and with infusion of fructose at two dosages (low F and high F). **A:** Rates of GP during the euglycemic study period (■) and the hyperglycemic study period (□) for the type 2 diabetic subjects with infusion of fructose at two dosages (low and high F) relative to their respective control studies ( $F^-$ ). **B:** Rates of GP during the euglycemic study period (■) and the hyperglycemic study period (□) for the nondiabetic subjects under the three experimental conditions.

the defective response of GP to hyperglycemia per se, the type 2 diabetic subjects manifested reduced effectiveness of glucose to enhance peripheral glucose uptake.

The coinfusion of fructose during the hyperglycemic study interval did not alter the response of glucose uptake to hyperglycemia in either the type 2 diabetic study groups (percent change in glucose uptake with hyperglycemia:  $53 \pm 12\%$  for low F vs.  $48 \pm 4\%$  for  $F^-$ ,  $53 \pm 8\%$  for high F vs.  $46 \pm 21\%$  for  $F^-$ ;  $P > 0.05$  vs.  $F^-$ ) or the nondiabetic study groups (percent change in glucose uptake with hyperglycemia:  $75 \pm 14\%$  for low F vs.  $82 \pm 14\%$  for  $F^-$ ,  $105 \pm 14\%$  for high F vs.  $96 \pm 27\%$  for  $F^-$ ;  $P > 0.05$  vs.  $F^-$ ) (Fig. 3A and B).

The glucose uptake estimates are derived from the total rate of disappearance of [ $3\text{-}^3\text{H}$ ]glucose from plasma, and would thus include both hepatic and peripheral glucose uptake. However, Cherrington et al. (48) reported that hyperglycemia does not significantly increase glucose uptake by the liver in the absence of changes in pancreatic hormones (i.e., increased insulin and/or decreased glucagon) or a "portal signal." Because the current studies were performed in fasting subjects with peripheral delivery of

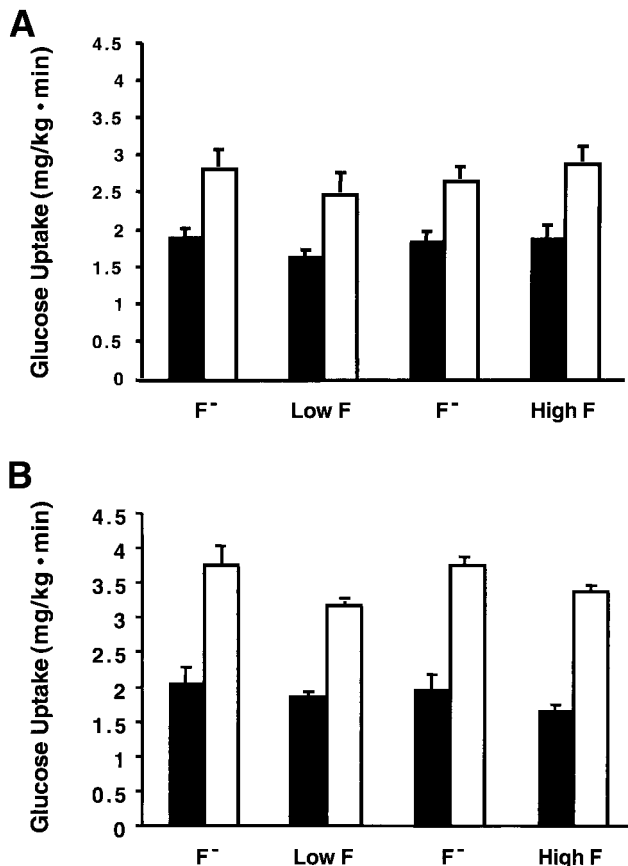


FIG. 3. Rates of glucose uptake during euglycemic-hyperglycemic studies in the type 2 diabetic and nondiabetic subjects, without (F<sup>-</sup>) and with infusion of fructose at two dosages (low and high F). A: Rates of glucose uptake during the euglycemic study period (■) and the hyperglycemic study period (□) for the type 2 diabetic subjects under the three experimental conditions. B: Rates of glucose uptake during the euglycemic study period (■) and the hyperglycemic study period (□) for the nondiabetic subjects under the three experimental conditions.

hormones and substrates under pancreatic clamp conditions, a fructose-induced increase in hepatic glucose uptake would not be sufficient to significantly enhance whole-body glucose disposal. Although fructose infusion was able to partially restore glucose effectiveness on GP, presumably via its effect on GK activation, peripheral glucose effectiveness was completely unaffected.

## DISCUSSION

The ability of hyperglycemia per se to regulate endogenous GP is markedly impaired in type 2 diabetes. This defect contributes importantly to fasting hyperglycemia in these individuals. Given the important role of GK in determining rates of GP, we examined the effect of stimulating GK on the control of glucose fluxes in nondiabetic and type 2 diabetic individuals. Rates of GP were measured under fixed hormonal conditions (somatostatin infusion and basal hormone replacement) in response to a standard (+90 mg/dl) increase in plasma glucose concentration. Although the rapid induction of hyperglycemia resulted in ~50% reduction in rates of GP in nondiabetic subjects, there was a complete lack of suppression in type 2 diabetic subjects. Given evidence for defects in hepatic glucose phosphorylation in type 2 diabetes (17,18), we examined whether stimulating GK activity with small

amounts of fructose (29,30,33) might restore glucose-induced inhibition of GP in type 2 diabetic subjects. Indeed, infusion of two doses of fructose partially restored the ability of glucose to suppress GP in type 2 diabetic subjects. In contrast, there was no additional suppression of GP by hyperglycemia with infusion of fructose in age- and weight-matched nondiabetic subjects. Furthermore, the addition of fructose did not affect rates of peripheral glucose uptake in either subject group.

The repeated-measures experimental design, in which each subject acted as his or her own control, was chosen to eliminate interindividual differences in rates of glucose fluxes (49). Indeed, metabolic parameters were remarkably stable (HbA<sub>1c</sub>, baseline plasma insulin, and FFA levels) in each individual between study days, as were initial (euglycemic) rates of GP and glucose uptake on separate days. Given the small intraindividual differences, the sample sizes in both groups yielded sufficient power to reject the null hypotheses for both study parameters (differences in GP and glucose uptake between euglycemia and hyperglycemia) (49).

Studies from our group have indicated that an acute increase in the plasma glucose concentration per se fails to decrease GP in both 90% pancreatectomized diabetic rats and humans with type 2 diabetes (5,10). Indeed, among humans with type 2 diabetes, the degree of impairment in the regulation of GP by hyperglycemia is proportional to their degree of glycemic control, and this defect is completely reversible with acute (72-h) restoration of optimal glycemia (50). Thus, chronic hyperglycemia and/or other metabolic derangements characteristic of type 2 diabetes may contribute to this loss of autoregulation, suggesting that the defect is at least partially acquired.

We reasoned that this inability to adapt endogenous GP to changes in plasma glucose concentration may be in part the consequence of impaired GK activity in vivo. To examine the effect of such a chronic defect in hepatic glucose phosphorylation, we had previously compared the response of hepatic glucose fluxes to a hyperglycemic challenge (+150 mg/dl) in two transgenic mouse models with a disrupted GK allele. One model involved a selective disruption of the GK allele in the pancreatic  $\beta$ -cells (RIP-GKRZ) (51), whereas the other model involved both liver and pancreas (GK<sup>+/-</sup>) (52). In the presence of fixed pancreatic hormone levels, hyperglycemia inhibited GP by only 12% in GK<sup>+/-</sup>, versus 42 and 45%, respectively, in the wild-type and RIP-GKRZ mice (16). Thus, the marked impairment in the ability of hyperglycemia to inhibit GP in the GK<sup>+/-</sup> animals is due to the specific decrease in hepatic GK activity. Acute partial inhibition of GK by glucosamine resulted in a similar loss of regulation of GP by hyperglycemia per se in normal rats (15). Also, defective GK activity contributes to the pathogenesis of abnormal hepatic glucose metabolism and ultimately of diabetes in MODY (17), and it contributes to a worsening metabolic picture in type 2 diabetes in general (18,19). These examples of acute and chronic decreases in hepatic GK activity all suggest a central role for GK in the regulation of hepatic glucose metabolism. Indeed, an impaired ability of hyperglycemia to increase hepatic glucose phosphorylation and

to inhibit GP probably contributes to fasting hyperglycemia in type 2 diabetes.

In the present studies, activation of glucose phosphorylation by catalytic amounts of fructose in human subjects provided a means of examining the role of hepatic GK activity in the regulation of endogenous GP by hyperglycemia in human type 2 diabetes. Indeed, increasing GK activity by various means has been demonstrated to improve glycemia and hepatic glucose regulation in several animal models of diabetes. Overexpression of hepatic GK not only prevented the fall in hepatic G-6-P levels in streptozotocin-induced diabetes but also normalized rates of hepatic gluconeogenesis and overall hepatic GP, and it significantly improved plasma glucose levels (53). GK overexpression restored normal rates of glycogen synthesis and several parameters of glucose utilization in cultured hepatocytes from ZDF rats (54). In addition, a modest ~20% elevation in hepatic GK activity in a nondiabetic transgenic mouse model resulted in decreased fasting glucose and insulin levels, and it blunted the rise in plasma glucose after a glucose tolerance test (55).

Moreover, acutely increasing GK translocation and hence glucose phosphorylation has had similar beneficial effects. Indeed, the administration of fructose either intraduodenally or intraportally in normal dogs resulted in a shift from net hepatic output to net hepatic uptake of glucose (29,30), and oral administration of fructose in both type 2 diabetic and nondiabetic humans significantly reduced the area under the curve for glucose after an oral glucose tolerance test (56,57). We have now demonstrated that acute stimulation of GK by catalytic concentrations of fructose restores the regulation of glucose fluxes by hyperglycemia per se in humans with type 2 diabetes. Because stimulatory effects of glucose on translocation and activation of GK contribute to the effect of hyperglycemia per se on GP in normal subjects (10), it is interesting to note that the type 2 diabetic subjects apparently lacked this stimulatory effect of glucose on hepatic GK and yet were responsive to fructose. Of note, the stimulatory effect of fructose on the translocation of GK is far more potent than that of glucose (26). Hence, it is very plausible that hyperglycemia alone was insufficient to activate the diminished pool of GK in the type 2 diabetic subjects, but that fructose was able to have an impact. However, it is also possible that the stimulatory effect of glucose itself persists in type 2 diabetic subjects, but that the desensitizing effects of chronic hyperglycemia are such that much higher plasma glucose levels would be required to observe the effect.

Because the current studies were designed to primarily examine glucose effectiveness and minimize the total dose of fructose given, fructose was only administered during hyperglycemia. Although the experimental design was therefore unable to rule out a direct effect of fructose on GP, the following arguments suggest that the major effect of fructose was to increase glucose effectiveness. In support of the potentiating effect of fructose on hepatic glucose effectiveness is the recent model of GK activation proposed by Moukil and Van Schaftingen (8), in which the GK enzyme slowly transitions between low- and high-affinity forms. After the release of phosphorylated substrate from the catalytic region of GK, the enzyme

transiently remains in a high-affinity state such that if an abundance of substrate is available, it will be more avidly phosphorylated by GK. This “cooperative” model would imply that fructose is likely to exert more pronounced effects on the G-6-P pool and hence on the regulation of GP in the presence of hyperglycemia. Furthermore, two rodent models of decreased GK activity provide in vivo evidence that the impact of GK stimulation on GP is most important in the presence of hyperglycemia. Both acute inhibition of GK with glucosamine infusion (15) and a targeted disruption of the GK gene (16) resulted in an inability to suppress GP in response to hyperglycemia, without any alterations in euglycemic rates of GP under basal, fixed hormonal conditions.

Besides the restorative effects of fructose on the regulation of total GP by hyperglycemia per se, other effects of fructose on hepatic glucose metabolism might be of potential therapeutic benefit in type 2 diabetes. Consistent with the important interrelationship between GK translocation and activation of glycogen synthase (31,32), fructose acutely increases rates of glycogen synthesis in vitro in hepatocytes (58) and in vivo in normal dogs (29) and humans (33). Because the liver’s ability to store glucose as glycogen is decreased in type 2 diabetes (59), this effect of fructose is likely to be of additional therapeutic benefit. Furthermore, fructose administration increases rates of hepatic glycolysis, resulting in increased lactate production (54,58). This fructose-induced stimulation of glycolysis is likely due to increased fructose-2,6-P<sub>2</sub> levels (60)—probably secondary to the effect of fructose on glucose phosphorylation (61)—and to increased activity of pyruvate kinase (53,62). Indeed, the increased plasma lactate levels observed with fructose infusion in these studies likely reflect stimulation of hepatic glycolysis.

As noted above, the infusion rates of fructose were carefully selected to be “catalytic” for GK translocation while avoiding potential adverse effects of higher doses. Plasma fructose levels of ~1 mmol/l provide increased substrate for gluconeogenesis (39), although such increases in gluconeogenesis failed to affect overall rates of GP in either nondiabetic or type 2 diabetic subjects (39,63). Chronic ingestion of large amounts of fructose, totaling 10–20% of overall caloric intake, was associated with increased circulating triglyceride levels in numerous clinical studies (64). Indeed, marked overexpression of hepatic GK by means of a recombinant adenovirus, attaining 6.4-fold elevations in expression in fed rats, lowered plasma glucose and insulin levels at the expense of marked elevations in plasma FFAs and triglycerides (65). Clearly, the impact of this dramatic level of hepatic GK overexpression on glucose phosphorylation would far exceed any modest stimulation of GK attained with the current doses of fructose. In fact, such an adverse effect on plasma lipids was not observed in transgenic models involving more modest increases in GK activity (55,65).

Of note, although both doses of fructose infusion significantly improved the effectiveness of glucose to suppress GP in the type 2 diabetic subjects, neither was able to fully restore the degree of suppression observed in the nondiabetic subjects. Thus, the chronic inhibitory effects of the diabetic milieu on GK gene expression may be such that acutely stimulating a reduced pool of GK enzyme is not

sufficient to fully restore total GK activity. Indeed, the comparable effects with both infusion rates suggest that plasma levels of  $\sim 100 \mu\text{mol/l}$  were sufficient for full stimulatory effects on GK translocation.

Alternatively, the failure of fructose to restore normal regulation might emphasize the complementary role of glucose-6-phosphatase (G-6-Pase) in determining responses of hepatic glucose fluxes to hyperglycemia per se. Indeed, fructose does not affect G-6-Pase activity (66,67), which is elevated acutely by hyperglycemia (68–71) and chronically in type 2 diabetes (18,19). As noted above, hepatic “autoregulation” of GP by hyperglycemia probably resides at the level of the G-6-P pool, which is tightly regulated by the relative glucose flux through both GK and G-6-Pase. Because increased expression of G-6-Pase results in elevated rates of GP even in the absence of hyperglycemia (72), this could clearly contribute to the abnormally elevated rates of GP in type 2 diabetes and the lack of suppression with hyperglycemia.

Although administration of fructose partially restored the suppression of hepatic GP by hyperglycemia in the type 2 diabetic subjects, there was no effect on peripheral glucose uptake in either the type 2 diabetic subjects or the nondiabetic group. This might be predicted from known differences in the regulation of the isozymes of hexokinase. Whereas the activity of GK (hexokinase IV) is regulated by the affinity of its binding to a specific inhibitory protein, as described above, the other isozymes do not share this mechanism of regulation (58). Hence, fructose would not be expected to alter the phosphorylation of incoming glucose in peripheral tissues.

In summary, the infusion of relatively small amounts of fructose in moderately controlled type 2 diabetic subjects partially corrected the regulation of GP by hyperglycemia per se. This suggests that an impaired ability of glucose to stimulate flux through GK ultimately contributes to increased GP in individuals with type 2 diabetes. Thus, activating the translocation of hepatic GK offers an attractive treatment option to restore glucose-induced regulation of hepatic glucose fluxes. Given the beneficial effects of GK overexpression in an insulin-deficient, streptozotocin-induced mouse model of diabetes (53) and the fact that defective regulation of hepatic glucose fluxes in diabetes is determined by the degree of chronic hyperglycemia (50), administration of fructose might be expected to favorably affect hepatic glucose metabolism in type 1 diabetes as well.

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