

Fructose Amplifies Counterregulatory Responses to Hypoglycemia in Humans

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Glucokinase (GK) is required for cellular glucose sensing, although there is a paucity of data regarding its role in the counterregulatory response to hypoglycemia in humans. Because fructose has been shown to modulate GK activity, we examined the effects of an acute infusion of fructose on hypoglycemia counterregulation in seven lean nondiabetic subjects. Using stepped hypoglycemia clamp studies (5.0, 4.4, 3.9, and 3.3 mmol/l target plasma glucose steps, 50 min each), subjects were studied on two separate occasions, without (control) or with co-infusion of fructose ($1.2 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$). Fructose induced a resetting of the glycemic thresholds for secretion of epinephrine ($3.8 \pm 0.1 \text{ mmol/l}$) and glucagon ($3.9 \pm 0.2 \text{ mmol/l}$) to higher plasma glucose concentrations ($4.0 \pm 0.1 \text{ mmol/l}$ [$P = 0.006$] and $4.1 \pm 0.1 \text{ mmol/l}$ [$P = 0.03$], respectively). In addition, the magnitude of increase in epinephrine and glucagon concentrations was higher after administration of fructose (48 and 39%, respectively, $P < 0.05$ for both). The amplification of these hormonal responses was specific because plasma norepinephrine, growth hormone, and cortisol were comparable in both sets of studies. Endogenous glucose production, measured with [$3\text{-}^3\text{H}$]glucose, increased by 47% ($P < 0.05$) in the fructose infusion studies compared with 14% ($P = \text{NS}$) in the control studies. In addition, glucose uptake was more suppressed with fructose infusion (by 33%, $P < 0.05$). In concert with these effects of fructose on glucose kinetics, average glucose infusion rate was markedly reduced in the fructose infusion studies during the 3.9-mmol/l glucose step (4.6 ± 0.9 vs. $7.4 \pm 1.1 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, respectively, $P = 0.03$) and during the 3.3-mmol/l glucose step (0.5 ± 0.1 vs. $5.2 \pm 1.2 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, respectively, $P < 0.001$), suggesting more potent glucose counterregulation and improved recovery from hypoglycemia with fructose infusion. We conclude that infusion of a catalytic dose of fructose amplifies the counterregulatory response to hypoglycemia by both increases in hormonal activation and augmentation of glucose counterregulation in humans. *Diabetes* 51: 893–900, 2002

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CNS, central nervous system; EGP, endogenous glucose production; FK, fructokinase; GCRC, General Clinical Research Center; GK, glucokinase; GKR, GK regulatory protein; HNF-1 α , hepatocyte nuclear factor-1 α ; MODY, maturity-onset diabetes of the young; VMH, ventro-medial hypothalamus.

Induction of hypoglycemia typically generates a counterregulatory response characterized by release of counterregulatory hormones and activation of endogenous glucose production (EGP), which ultimately results in a recovery in plasma glucose (1). The initiation of secretion of each counterregulatory hormone occurs at a specific plasma glucose level, or “glycemic threshold” (1–3). Glucagon and epinephrine are the major counterregulatory hormones that contribute to glucose counterregulation during moderate hypoglycemia; both activate EGP in order to overcome the insulin-induced suppression of glucose formation/release (3,4). Patients with type 1 diabetes suffer from an altered counterregulatory hormonal response to hypoglycemia characterized by severe blunting or absence of the glucagon response and both a lowered glucose threshold for and a reduced magnitude of epinephrine secretion (5,6). As a consequence, these patients have an increased risk of developing severe hypoglycemia (7–11).

Central nervous system (CNS) signals that mediate the response to hypoglycemia may be of major importance in glucose counterregulation, and defective sensing of hypoglycemia in type 1 diabetes may represent a fundamental alteration causing inadequate recovery from hypoglycemia in these patients (rev. in 11). Glucokinase (GK), the enzyme that specifically catalyzes glucose phosphorylation, is considered to be the primary glucose sensor in pancreatic β -cells and in the liver (13,14). Recent studies, however, have demonstrated GK mRNA in other locations that have been implicated in glucose sensing, such as the paraventricular and ventro-medial hypothalamic (VMH) nuclei in the brain (15,16).

GK activity is acutely regulated by its interaction with GK regulatory protein (GKR). The regulatory protein located within the nucleus functions as an anchor, binding and inhibiting GK (17–19). It has been shown that the regulatory protein is activated by fructose-6-phosphate and suppressed by fructose-1-phosphate (20,21). Van Shaftingen et al. (21–23) and Agius et al. (19) have shown that the addition of catalytic amounts of fructose increases fructose-1-phosphate in the hepatocyte and promotes the release of GK from its regulatory protein, which results in GK activation. The phosphorylation of fructose into fructose-1-phosphate requires fructokinase (FK), which is present in hepatocytes. However, in glucose sensing cells located in the CNS (which lack FK), fructose can still be

phosphorylated (via hexokinase) into fructose-6-phosphate, thus generating an inhibitory effect on GK activity (22).

The potential use of fructose as a tool to augment the counterregulatory response to hypoglycemia is based on two hypotheses. First, we reasoned that inhibition of GK during hypoglycemia could affect the set point for hypoglycemia sensing. Second, it has been recently demonstrated that administration of a small dose of fructose induces an increase in hepatic glycogen synthase activity, which may also contribute to a modulatory effect of fructose on counterregulation by increasing net hepatic glycogen content (for ultimate phosphorylation) (24). Thus, we examined whether fructose-induced modulation of GK during hypoglycemia could sensitize the putative hypoglycemic sensor to impending hypoglycemia and consequently augment the counterregulatory response in normal subjects and/or modulate EGP by enhancing the response to hypoglycemia. We used a well-established experimental approach in humans—the stepped hypoglycemic clamp—in conjunction with [^3H]glucose to trace glucose fluxes in paired studies with either fructose or saline control infusion.

RESEARCH DESIGN AND METHODS

We studied seven lean nondiabetic volunteers (five men and two women, aged 33 ± 3 years, BMI 24.2 ± 1.42 kg/m 2 , HbA $_{1c}$ $5.9 \pm 0.2\%$). All participants were in good health, were not taking any medications, and had no family history of diabetes. Each subject participated in two stepped hypoglycemic clamp studies separated by an interval of 6 weeks. During each hypoglycemic clamp, subjects received either fructose (1.2 mg \cdot kg $^{-1}$ \cdot min $^{-1}$) or saline intravenously in random order. Clamp studies were performed after an overnight fast.

Informed written consent was obtained in accordance with policy of the Committee on Clinical Investigations of the Albert Einstein College of Medicine.

Procedures. Subjects were admitted to the General Clinical Research Center (GCRC) for each experiment. At 0700 on the day of study, two indwelling cannulae were inserted, one in an antecubital vein for infusions and the second placed in a retrograde fashion in a distal hand vein of the contralateral forearm for blood sampling. To obtain arterialized venous blood samples, this hand was maintained at 55°C in a thermoregulated Plexiglas box. At $t = -120$ min, a primed-continuous infusion of HPLC-purified [^3H]glucose was initiated with a bolus of 21.6 μCi followed by continuous infusion of 0.15 $\mu\text{Ci}/\text{min}$ for the entire period of study.

The specific activity of infused dextrose was kept equivalent to plasma glucose specific activity by addition of [^3H]glucose to the infusate, using the method of Finegood et al. (25) to prevent negative R_a artifacts. At $t = 0$ min, a primed-continuous infusion of insulin (Humulin Regular; Eli Lilly, Indianapolis, IN) at a rate of 0.4 mU \cdot kg $^{-1}$ \cdot min $^{-1}$ was initiated, and a variable infusion of 20% dextrose was begun to maintain the plasma glucose concentration at 5.0 mmol/l for 50 min (step one of the clamp). At $t = +50$ min and every 50 min thereafter, the plasma glucose concentration was decreased by 0.5 mmol/l decrements for 50 min, each by reducing the dextrose infusion rate accordingly. The plasma glucose was clamped at the desired range by varying the dextrose infusion according to plasma glucose measured at 5-min intervals with targets of 4.4, 3.9, and 3.3 mmol/l. At $t = +50$ min (plasma glucose ~ 5.0 mmol/l), an infusion of fructose 1.2 mg \cdot kg $^{-1}$ \cdot min $^{-1}$ or saline was initiated and maintained constant until the end of the study. This infusion rate of fructose was selected in order to achieve plasma fructose levels of ~ 200 $\mu\text{mol/l}$ (26,27). Such low-circulating levels of fructose would be regulatory for GK translocation, without providing increased substrate for gluconeogenesis (28). However, to examine whether fructose infusion could directly influence the rates of glucose production or peripheral glucose uptake in the absence of changes in the counterregulatory hormones, we performed another set of studies in five healthy lean volunteers matched for age, BMI, and HbA $_{1c}$ with the participants in the stepped hypoglycemia studies (three men and two women, BMI 23.1 ± 2.81 kg/m 2 , HbA $_{1c}$ $5.4 \pm 0.5\%$). Using the pancreatic clamp technique to suppress glucagon secretion and lowering the plasma glucose to 4.2 mmol/l (above the expected glycemic threshold for other counterregulatory hormones secretion), we examined the effects of fructose per se on EGP. Glucose tracer was administered as previously described. 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 $^{-1}$ \cdot min $^{-1}$) (Genentech, San Francisco, CA), glucagon (1.0 ng \cdot kg $^{-1}$ \cdot min $^{-1}$) (Eli Lilly), and 1 mg/ml albumin diluted in saline was initiated and maintained throughout the study. Insulin (Humulin Regular) infusion was also initiated at $t = 0$ and maintained at a fixed rate of 0.4 mU \cdot kg $^{-1}$ \cdot min $^{-1}$. Plasma glucose was maintained at basal concentrations during the first 120 min by a variable infusion of dextrose 20% (i.e., the 5.0 mmol/l nominal glucose step). At $t = 120$ min, plasma glucose was lowered to 4.2 mmol/l by decreasing the dextrose infusion rate, and simultaneously an infusion of either fructose (1.2 mg \cdot kg $^{-1}$ \cdot min $^{-1}$) or saline was initiated and maintained for the final 120 min of the study (the 4.2-mmol/l nominal glucose step). Thus, by suppressing glucagon and growth hormone secretion (with somatostatin) and maintaining the plasma glucose above the threshold for counterregulatory hormone secretion, the direct effect of fructose (versus saline) on glucose counterregulation was assessed. At the end of the clamp, all of the infusions were discontinued, and the subject was given a meal and discharged from the GCRC.

Fasting blood for HbA $_{1c}$ was collected on the morning of each clamp study. During the clamps, blood samples were obtained for the determinations of plasma insulin, C-peptide, glucagon, epinephrine, norepinephrine, cortisol, growth hormone, and lactate, as well as for glucose kinetics.

Analytical methods. Plasma glucose was measured with a Beckman glucose analyzer (Fullerton, CA), using the glucose oxidase method. Plasma [^3H]glucose radioactivity was measured in duplicate on the supernatants of barium hydroxide-zinc sulfate precipitates of plasma samples after evaporation to dryness to eliminate tritiated water.

The methods for measurement of plasma insulin, C-peptide, glucagon, epinephrine, norepinephrine, cortisol, growth hormone, lactate, and their intra- and interassay variations have been previously reported (29).

Analyses. The data in the text, figures, and tables are presented as means \pm SE. Steele's equation was used for calculation of glucose turnover as described elsewhere (30). Values for EGP and glucose uptake, obtained at 10-min intervals, were averaged over the final 30 min of each glucose step for each individual subject. The glycemic thresholds for activation of counterregulatory hormone secretion was calculated as the glycemic level at which there was an increase of >2 SDs above the basal plasma hormone concentration (31,32). The area under curve was calculated using the trapezoidal method for the whole duration of the clamp (31). Statistical analyses were performed using repeated measures ANOVA for multiple comparisons and paired Student's t test for comparing means before and after the intervention (fructose infusion). A value of $P < 0.05$ was considered significant.

RESULTS

Stepped hypoglycemia clamp studies

Plasma glucose levels and glucose infusion rates.

Plasma glucose concentrations during both clamps (fructose and control) are shown in Fig. 1A. Plasma glucose concentrations at $t = 0$ min were 5.1 ± 0.1 mmol/l in the fructose studies and 4.9 ± 0.1 mmol/l in the control studies ($P = \text{NS}$). No significant difference was noted between the fructose and control studies during the 5.0-, 4.4-, or 3.9-mmol/l plasma glucose steps (4.9 ± 0.1 , 4.6 ± 0.1 , and 4.0 ± 0.1 mmol/l, respectively, in the fructose studies and 4.9 ± 0.1 , 4.5 ± 0.1 , and 3.9 ± 0.1 mmol/l, respectively, in the control studies). However, during the 3.3 mmol/l plasma glucose step, there was a significantly higher plasma glucose concentration in the fructose studies compared with control studies (3.8 ± 0.1 vs. 3.4 ± 0.1 mmol/l, respectively, $P = 0.009$). The higher plasma glucose levels in the fructose studies during this glucose step occurred despite the fact that the glucose infusion was discontinued and coincided with elevated plasma concentration of epinephrine and glucagon. Thus, using the same insulin infusion rate (0.4 mU \cdot kg $^{-1}$ \cdot min $^{-1}$) in the fructose and control studies, plasma glucose could not be lowered to the desired glucose step in the fructose studies, confirming that the glucose counterregulatory systems were activated.

Glucose infusion rates in the experimental protocol are depicted in Fig. 1B. During the first and second glucose

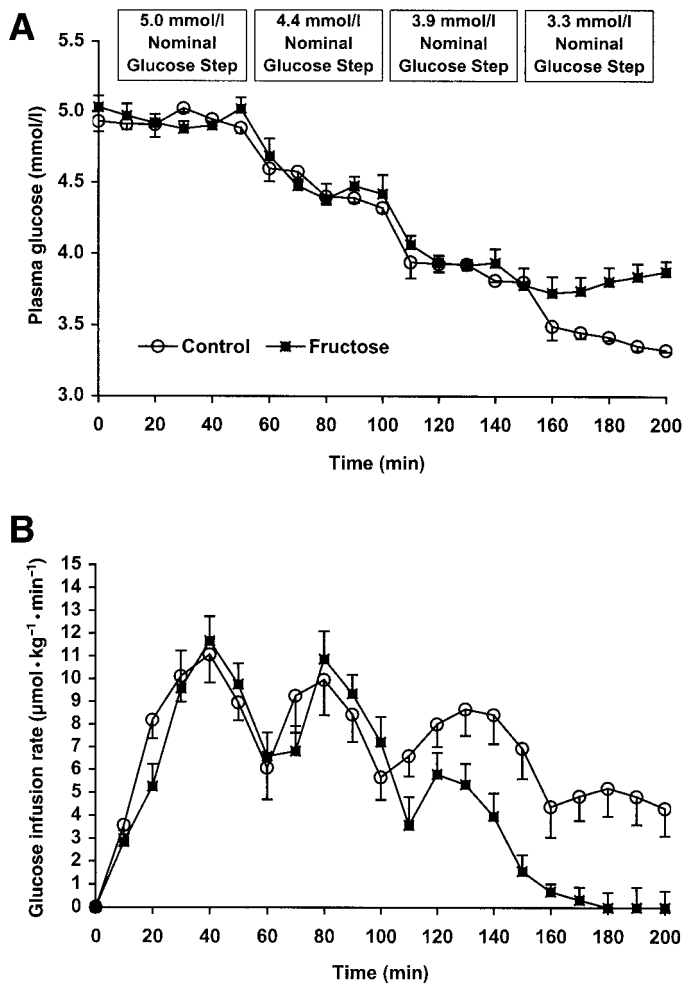


FIG. 1. A: Plasma glucose concentrations at each glucose step with and without co-infusion of fructose. B: Glucose infusion rates over time fructose and control studies.

steps, average glucose infusion rates were comparable in both studies (7.2 ± 1.1 and $7.7 \pm 1.1 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ in the fructose and control studies, respectively, $P = \text{NS}$). However, during the 3.9-mmol/l glucose step, the mean rate of glucose infusion was lower in the fructose studies (4.6 ± 0.9 vs. $7.4 \pm 1.1 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, $P = 0.03$), and during the 3.3-mmol/l glucose step, the average glucose infusion rate was 0.5 ± 0.1 in the fructose studies versus $5.2 \pm 1.2 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ in the control studies ($P < 0.001$). It is important to emphasize that during the final 20 min of the clamp ($t = 180$ – 200 min), glucose infusion was stopped in all fructose studies because plasma glucose concentration was above the desired (3.3 mmol/l) glucose step.

Plasma insulin and C-peptide concentrations. Basal plasma insulin concentrations were nearly identical in both studies, averaging 24.6 ± 7.2 pmol/l in the fructose studies and 30.6 ± 13.2 pmol/l in the control studies ($P = \text{NS}$). Similarly, there was no significant difference in plasma insulin concentration during both clamps, averaging 186.6 ± 8.4 pmol/l in the fructose studies and 180 ± 6.8 pmol/l in the control studies (Fig. 2A). Plasma C-peptide concentrations were comparable in both sets of studies at baseline (0.49 ± 0.06 nmol/l in the fructose studies and 0.52 ± 0.06 pmol/l in the control studies, $P = \text{NS}$) and

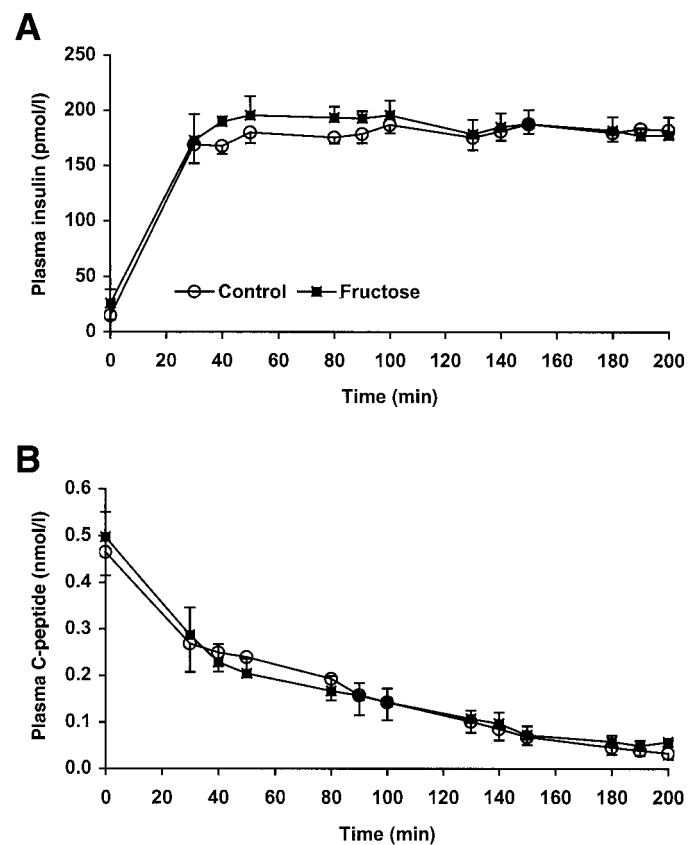


FIG. 2. Plasma insulin concentrations (A) and C-peptide concentrations (B) in the fructose and control studies.

remained very similar during the clamp, suppressing in both studies to 0.03 ± 0.01 pmol/l at the hypoglycemic nadir, at $t = 200$ min (Fig. 2B).

Counterregulatory hormones. During the 5.0- and 4.4-mmol/l glucose steps, plasma epinephrine concentrations remained near or at basal values and were similar in both fructose and control studies (193 ± 6 pmol/l with fructose and 219 ± 14 pmol/l without fructose, $P = \text{NS}$ [Table 1]). Further reduction in plasma glucose to 3.9 mmol/l was associated with an increment in plasma epinephrine in both sets of studies, though the threshold for epinephrine release occurred at a higher plasma glucose concentration in the fructose than in the control studies (4.0 ± 0.1 vs. 3.8 ± 0.1 mmol/l, respectively, $P = 0.006$) (Fig. 3A).

TABLE 1

Plasma epinephrine, glucagon, norepinephrine, cortisol, and growth hormone at baseline and averaged for the final 30 min of the 3.3-mmol/l glucose step in the stepped hypoglycemia studies

	Fructose		Control	
	Baseline	3.3 mmol/l	Baseline	3.3 mmol/l
Epinephrine (pmol/l)	219 ± 14	$3958 \pm 448^*$	193 ± 6.5	2784 ± 333
Glucagon (ng/l)	54 ± 12	$185 \pm 14^*$	67 ± 16	133 ± 17
Norepinephrine (nmol/l)	0.7 ± 0.3	1.5 ± 0.2	0.8 ± 0.3	1.2 ± 0.2
Cortisol (nmol/l)	252 ± 52	539 ± 45	306 ± 34	556 ± 68
Growth hormone ($\mu\text{g/l}$)	1.4 ± 0.9	18.2 ± 5.9	1.6 ± 0.6	15.2 ± 6.8

Data are means \pm SE. * $P < 0.05$ vs. control.

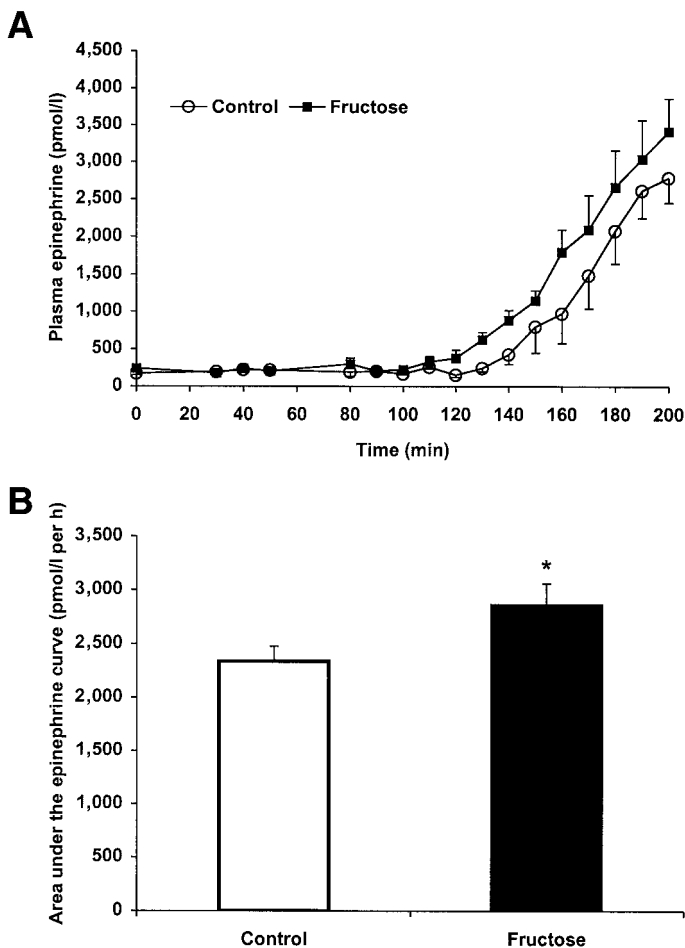


FIG. 3. Plasma epinephrine concentrations over time (A) and averaged for the area under the epinephrine curve (B) for studies with or without co-infusion of fructose. * $P < 0.05$ vs. control.

Moreover, the average maximal increase in plasma epinephrine was also significantly higher during the 3.9- and 3.3-mmol/l glucose steps in the fructose than in the control studies, equaling $1,152 \pm 131$ and 797 ± 93 pmol/l, respectively, during the 3.9 mmol/l glucose step, and $3,411 \pm 448$ pmol/l and $2,784 \pm 333$ pmol/l, respectively, during the 3.3 mmol/l glucose step (both $P < 0.05$). The area under the epinephrine curve during the whole duration of the clamps was also significantly higher in the fructose than in the control studies ($2,855 \pm 202$ vs. $2,336 \pm 142$ pmol \cdot l $^{-1}$ \cdot h $^{-1}$, $P < 0.05$) (Fig. 3B).

Plasma glucagon concentrations were equivalent during the 5.0- and 4.4-mmol/l glucose steps in both studies (Table 1). However, the threshold for glucagon release occurred at a significantly higher plasma glucose concentration in the fructose than the control studies (4.1 ± 0.1 and 3.9 ± 0.2 mmol/l, respectively, $P = 0.03$) (Fig. 4A). Similarly, the magnitude of glucagon release during the 3.9- and 3.3-mmol/l glucose steps was significantly higher in the fructose than in the control studies (117 ± 12 and 185 ± 14 ng/l in the fructose studies, respectively, and 97 ± 14 and 133 ± 17 ng/l in the control studies, respectively ($P < 0.05$ for both)). The area under the glucagon response curve was higher in the fructose than in the control studies (292 ± 28 vs. 215 ± 22 ng \cdot l $^{-1}$ \cdot h $^{-1}$, $P < 0.05$) (Fig. 4B).

In contrast to the amplified epinephrine and glucagon

responses in the fructose studies as compared with the control studies, plasma norepinephrine, growth hormone, and cortisol levels were similar in both sets of studies (Table 1).

Plasma lactate concentrations. Plasma lactate concentrations were similar in both studies and averaged 0.8 ± 0.1 and 0.8 ± 0.2 μ mol/l ($P = \text{NS}$) in the fructose and control studies, respectively, at baseline and 0.9 ± 0.1 and 1.1 ± 0.2 μ mol/l ($P = \text{NS}$) in the fructose and control studies, respectively, during the 3.3-mmol/l nominal glucose step.

Glucose kinetics. [$3\text{-}^3\text{H}$]glucose specific activity was effectively maintained in both sets of studies during the clamps (Table 2).

Mean fasting EGP was similar in the two studies (10.1 ± 1.1 and 10.5 ± 1.1 μ mol \cdot kg $^{-1}$ \cdot min $^{-1}$ in the fructose and control studies, respectively, $P = \text{NS}$). During the 5.0- and 4.4-mmol/l glucose steps (after the initiation of insulin infusion), EGP was equally suppressed by $\sim 45\%$ in both studies. During the next two clamp steps (3.9 and 3.3 mmol/l), EGP increased to 78 and 83%, respectively, of basal in the fructose studies and to 47 and 62%, respectively, in the control studies ($P < 0.05$ for both) (Fig. 5A), demonstrating a significant amplification of EGP in the fructose studies.

Glucose disposal rates were similar before insulin infu-

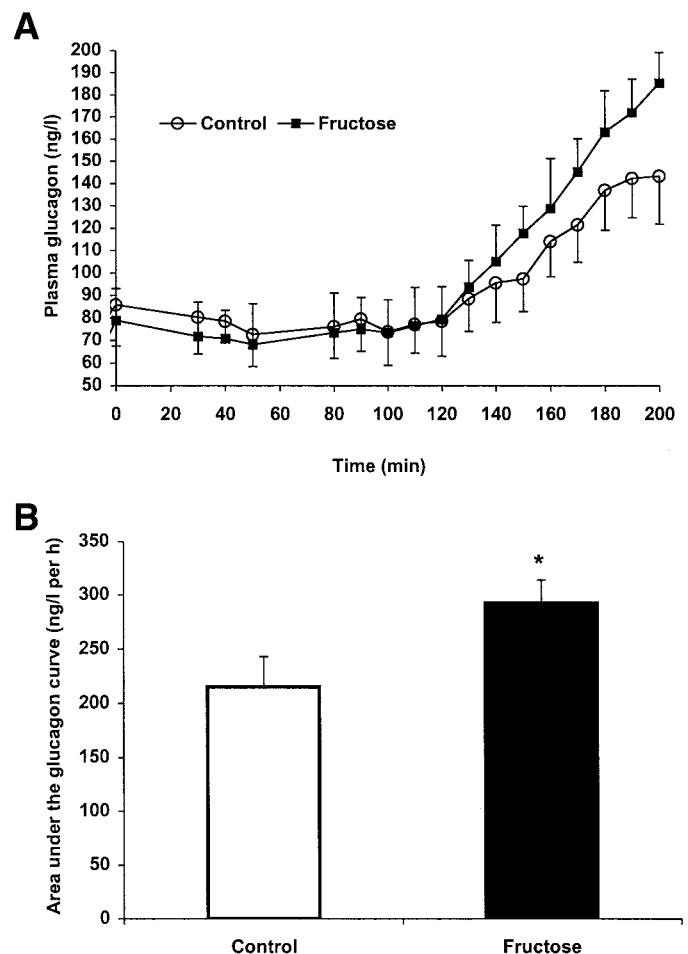


FIG. 4. Plasma glucagon concentrations over time (A) and averaged for the area under the glucagon curve (B) for studies with or without co-infusion of fructose. * $P < 0.05$ vs. control.

TABLE 2

Plasma [^3H]glucose specific activity in the stepped hypoglycemia studies at baseline and averaged for the final 30 minutes of each glucose step

Plasma specific activity (cpm/mg)	Nominal plasma glucose (mmol/l)				
	Baseline	5.0	4.4	3.9	3.3
Fructose	993 ± 92	976 ± 101	1022 ± 115	980 ± 75	993 ± 84
Control	922 ± 81	894 ± 62	941 ± 105	989 ± 63	950 ± 52

Data are means ± SE.

sion in all studies. With the initiation of the clamp, glucose disposal rates increased equally in the fructose and control studies by ~85% (to 20.1 ± 1.8 and $21.2 \pm 1.2 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, respectively, $P = \text{NS}$). Consequently, during the 3.9- and 3.3-mmol/l glucose steps, glucose disposal rates decreased by ~30% in both studies. However, the fructose studies demonstrated a more significant decrease in glucose uptake (R_d) than the control studies (11.8 ± 1.4 and $14.1 \pm 0.9 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, respectively, $P < 0.05$, during the 3.9-mmol/l glucose step and 12.8 ± 1.8 and $15.7 \pm 1.6 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, respectively, $P < 0.05$,

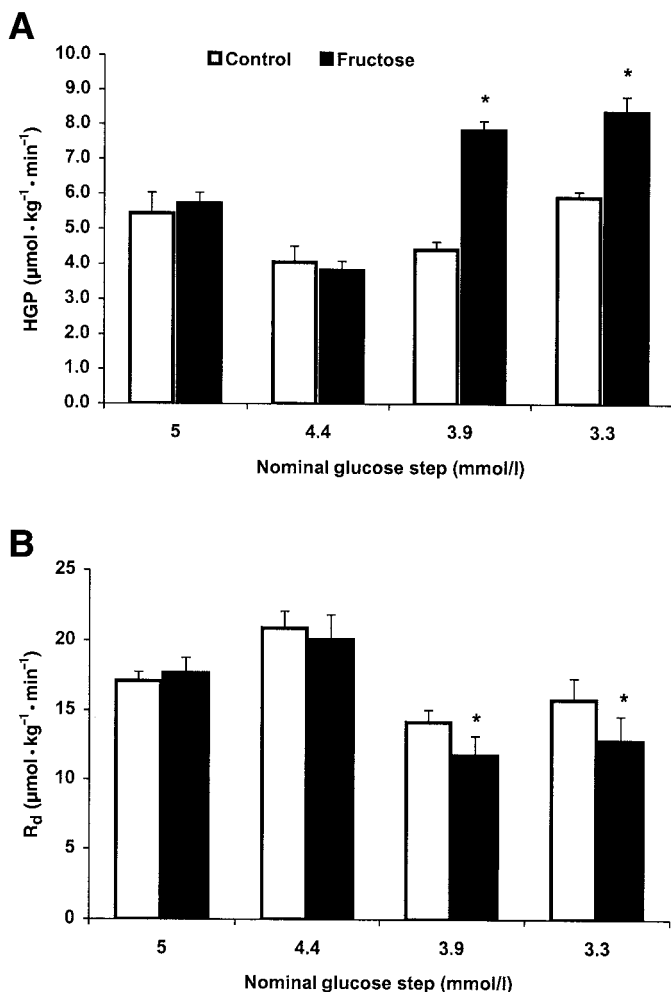


FIG. 5. EGP averaged for the final 30 min of each glucose step (A) and rates of glucose uptake (R_d) averaged for the final 30 min of each glucose step (B) for studies with or without co-infusion of fructose. * $P < 0.05$ vs. control.

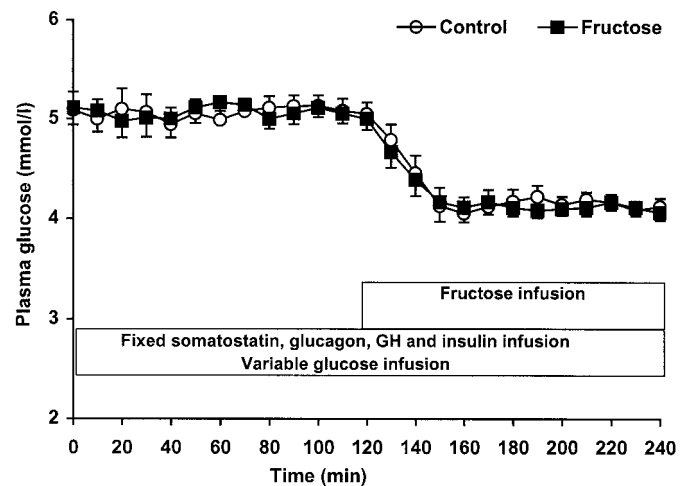


FIG. 6. Study protocol and plasma glucose concentrations during the pancreatic clamp studies with and without co-infusion of fructose.

during the 3.3-mmol/l glucose step) (Fig. 5B). It should be noted that the reduction in R_d during the fructose studies at the 3.3-mmol/l glucose step may be underestimated because plasma glucose was not lowered to the same level as in the control studies.

Pancreatic clamp studies

To exclude an effect of fructose on nonhypoglycemic hepatic glucose fluxes, we performed these studies at two plasma glucose steps: 5.0 and 4.2 mmol/l. Thus, we examined the effects of fructose on EGP excluding the action of the counterregulatory hormones. The study protocol and plasma glucose levels during both clamps (with and without co-infusion of fructose) were well maintained and similar during both clamps (Fig. 6). Mean plasma glucose concentrations at the nominal 5.0- and 4.2-mmol/l glucose steps were 5.2 ± 0.5 and 4.2 ± 0.2 mmol/l in the fructose studies and 5.1 ± 0.4 and 4.2 ± 0.1 mmol/l in the control studies, respectively ($P = \text{NS}$).

Plasma insulin, C-peptide, glucagon, epinephrine, norepinephrine, growth hormone, and cortisol concentrations were comparable in both clamps and are shown in Table 3.

Plasma lactate concentrations were similar in both studies and averaged 0.8 ± 0.2 and $0.7 \pm 0.1 \mu\text{mol/l}$ in the fructose and control studies, respectively, at baseline and 0.9 ± 0.2 and $0.8 \pm 0.3 \mu\text{mol/l}$ in the fructose and control studies, respectively, during the 4.2 mmol/l nominal glucose step.

The mean rates of EGP (Fig. 7) were similar at baseline (10.5 ± 0.9 and $10.6 \pm 0.5 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ for fructose and control studies, respectively, $P = \text{NS}$), during the nominal 5.0-mmol/l glucose step (5.2 ± 0.9 and $4.7 \pm 0.6 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ for fructose and control studies, respectively, $P = \text{NS}$), and during the nominal 4.2 mmol/l glucose step (3.9 ± 0.9 and $3.7 \pm 0.6 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ for fructose and control studies, respectively, $P = \text{NS}$). Glucose uptake was also comparable (Fig. 7) in both clamps at baseline (9.8 ± 1.2 and $10.1 \pm 0.8 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ for fructose and control studies, respectively, $P = \text{NS}$), during the nominal 5.0 mmol/l glucose step (15.7 ± 1.7 and $14.8 \pm 1.3 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ for fructose and control studies, respectively, $P = \text{NS}$), and during the nominal 4.2-mmol/l glucose step (14.9 ± 1.4 and 15.8 ± 1.6

TABLE 3

Plasma hormone concentrations during pancreatic clamp studies. Plasma insulin, C-peptide, epinephrine, glucagon, norepinephrine, cortisol, and growth hormone at baseline and averaged for the 5.0- and 4.2-mmol/l glucose steps

	Nominal plasma glucose (mmol/l)					
	Fructose			Control		
	Baseline	5.0	4.2	Baseline	5.0	4.2
Insulin (pmol/l)	31 ± 9	152 ± 4	168 ± 2	29 ± 7	163 ± 3	174 ± 4
C-peptide (nmol/l)	0.46 ± 0.09	0.03 ± 0.01	0.02 ± 0.01	0.49 ± 0.09	0.03 ± 0.01	0.02 ± 0.01
Epinephrine (pmol/l)	192 ± 17	244 ± 18	335 ± 48	157 ± 14	188 ± 15	302 ± 56
Glucagon (ng/l)	68 ± 10	74 ± 5	80 ± 6	65 ± 13	78 ± 7	72 ± 8
Norepinephrine (nmol/l)	0.7 ± 0.2	0.8 ± 0.2	0.9 ± 0.2	0.7 ± 0.2	0.9 ± 0.3	0.8 ± 0.3
Cortisol (nmol/l)	279 ± 33	317 ± 44	325 ± 55	243 ± 36	270 ± 44	295 ± 52
Growth hormone (μg/l)	1.5 ± 0.7	1.5 ± 0.9	2.2 ± 1.3	1.6 ± 0.4	1.6 ± 0.6	3.1 ± 1.8

Data are means ± SE.

μmol · kg⁻¹ · min⁻¹ for fructose and control studies, respectively, *P* = NS).

DISCUSSION

Our results demonstrate that intravenous infusion of catalytic amounts of fructose induce an amplification of the counterregulatory response to mild hypoglycemia in nondiabetic subjects. The augmentation of hormonal counterregulation was characterized by both a shift in the threshold for epinephrine and glucagon secretion (a significant release of these hormones occurred at a higher plasma glucose concentration) and a higher magnitude of epinephrine and glucagon release (as reflected by both peak and AUC concentrations) compared with matched control studies. These modestly augmented hormonal responses to hypoglycemia were accompanied by a markedly amplified EGP response and a relative greater reduction of glucose uptake. Indeed, because of this enhanced counterregulatory response in the fructose infusion studies, we were unable to lower the plasma glucose levels to <3.8 mmol/l. Thus, despite the same plasma insulin concentrations (~180 pmol/l in both studies), we could not attain the final glucose step (3.3 mmol/l target plasma glucose) in the fructose infusion studies, a factor that contributes to underestimation of the magnitude of the counterregulatory response in these studies but emphasizes the potency of the enhanced glucose counterregulation in preventing further hypoglycemia. The fact that the activation of EGP with fructose was disproportionately higher than the difference in the thresholds and magnitudes of epinephrine and glucagon release suggests that fructose may have an additional and independent effect on EGP during mild hypoglycemia. Thus, the fructose effect on glucose counterregulation in the stepped hypoglycemia studies could be attributed to the augmentation of epinephrine and glucagon responses and also to direct effect of hypoglycemia per se. Interestingly, the rates of EGP and glucose uptake did not change with fructose infusion (compared with saline) during the pancreatic clamp when the counterregulatory hormones were at baseline, although the plasma glucose levels in this protocol were clamped above the threshold for triggering hypoglycemia counterregulation.

Accumulating evidence suggests that the brain—in particular the VMH—plays an important role in glucose sensing. In dogs, cerebral perfusion of glucose abolishes

the counterregulatory response in the presence of peripheral hypoglycemia (33). In rats, selective destruction of the VMH by injection of ibotenic acid or localized perfusion of concentrated glucose solutions into the ventromedial nuclei abolishes the counterregulatory response (34,35). In contrast, selective glycopenia in the cells within the VMH by perfusion of 2-deoxyglucose activates counterregulation, even in the presence of normal circulating blood glucose concentration (36). Unfortunately, however, there are no data regarding these potential sites for hypoglycemia sensing and integration of the counterregulatory response in humans.

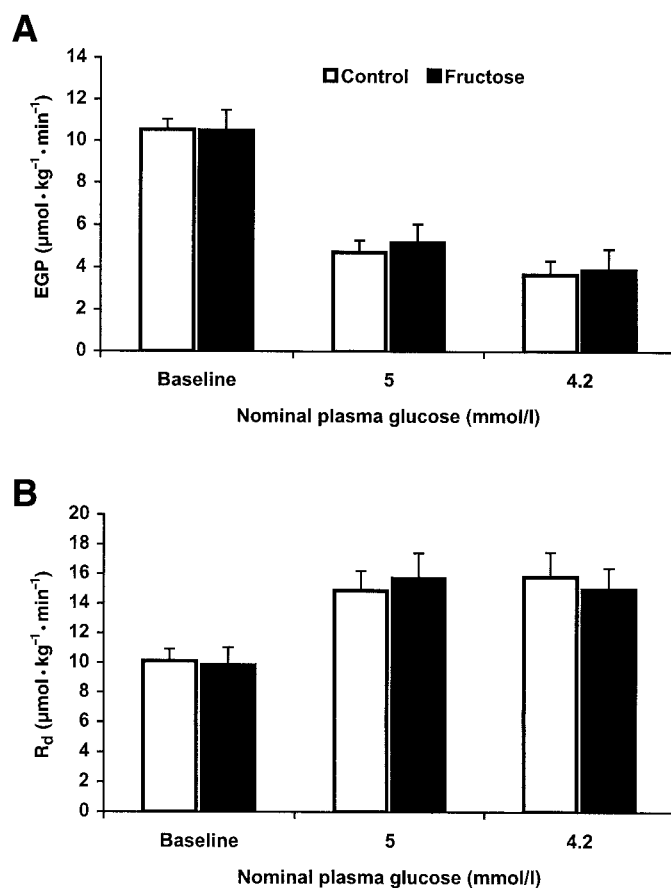


FIG. 7. EGP (A) and rates of glucose uptake (R_d) (B) during the pancreatic clamp at baseline and averaged for the 5.0- and 4.2-mmol/l glucose steps.

The mechanism by which fructose infusion might augment the counterregulatory response to hypoglycemia is not known. *In vitro* studies have shown that fructose-1-phosphate can activate glucokinase translocation by binding to the GKR. GKR inhibits GK in an allosteric manner that is competitive with respect to glucose. Conversely, fructose-6-phosphate binds to GKR and greatly enhances its binding to and inhibition of GK, whereas fructose-1-phosphate promotes the dissociation of this complex and activates GK. Under basal glucose conditions, GKR inhibits GK activity by its sequestration in the perinuclear area, thus rendering it inaccessible to incoming glucose. Fructose-1-phosphate, generated from fructose through the action of hepatic FK, binds to the regulatory protein and thereby decreases its affinity for GK. This alters the configuration of GK and permits its immediate translocation to the cytosol, both of which are responsible for increased enzyme activity. The operation of this rapid regulatory mechanism on EGP during hyperglycemia has been recently demonstrated in conscious dogs by administering small amounts of fructose either intraportally or intraduodenally (26,27). However, in cells that lack FK, GK phosphorylates fructose (via hexokinase) into fructose-6-phosphate, which may severely inhibit GK activity.

With respect to the role of GK in hypoglycemia, there is a paucity of published data the normal physiology in humans. Interestingly, patients with maturity-onset diabetes of the young (MODY)-II, whose mutation results in a GK protein with decreased activity in the β -cell (and presumably in the liver), appear to have an amplified counterregulatory response to low blood glucose, underscoring the potential role of GK in the defense against hypoglycemia (37). Specifically, in MODY II patients, Guenat et al. (37) reported higher glycemic thresholds for glucagon release and perhaps for EGP activation compared with nondiabetic subjects and patients with type 2 diabetes. These findings suggest that a partial decrease in GK activity in these patients may influence hypoglycemia counterregulation by acting at the level of hypoglycemia sensing because only the thresholds for (and not the magnitude of) glucagon and EGP were altered. The same authors performed similar studies in subjects with MODY III (hepatocyte nuclear factor-1 α [HNF-1 α] mutations) and demonstrated no significant difference in the counterregulatory response to hypoglycemia compared with nondiabetic subjects and patients with type 2 diabetes, suggesting that mutations of HNF-1 α in MODY III do not modulate counterregulatory responses to hypoglycemia (38).

We have used fructose as a pharmacological modulator of GK during hypoglycemia in nondiabetic subjects. An acute infusion of low-dose fructose induced a shift in the thresholds for epinephrine and glucagon release to a higher plasma glucose concentration and an increase in the magnitude of hormone secretion. Most importantly, glucose counterregulation (characterized by an increase in EGP) increased significantly and dramatically with fructose infusion. This remarkable effect of fructose infusion on hypoglycemia counterregulation could be explained, in part, by an apparent inhibitory action of fructose on GK activity in cells that lack FK (e.g., hypoglycemia-sensing cells in the hypothalamus). In support of this hypothesis, it

has been demonstrated that certain areas in the brain considered to be responsible for glucose sensing, such as the VMH, possess glucose-sensitive neurons that increase their firing rate when ambient glucose decreases (39). These neurons also possess the key biochemical elements required for glucose sensing (GK, GKR, and K_{ATP} channels) (16,40). Although fructose does not pass readily through the blood brain barrier, these hypothalamic glucose-sensitive neurons may be exposed to the plasma glucose concentrations prevailing in the systemic circulation and thus respond to physiological variations in plasma glucose concentration (39).

Another mechanism that may be responsible for the increase in EGP with fructose infusion during hypoglycemia is by transient activation of hepatic GK to increase the glycogen pool (G-6-P \rightarrow G-1-P) for further utilization. In support of this mechanism, Petersen et al. (24) have recently demonstrated that infusion of a low dose of fructose to nondiabetic subjects during a hyperinsulinemic-euglycemic clamp induces a threefold increase in net hepatic glycogen synthesis. Because net hepatic glycogen synthesis has been shown to be diminished in poorly controlled type 1 diabetic patients (41), previous activation of glycogen synthase and increasing net hepatic glycogen content with fructose infusion could positively contribute to EGP during subsequent hypoglycemia. Taken together, our data suggest that fructose has a potent modulatory effect on GK, inducing a significant amplification of the counterregulatory response to hypoglycemia in nondiabetic subjects. This effect may be attributed to fructose's action on the counterregulatory hormonal response and also to a potentially direct effect on EGP. Indeed, our results clearly demonstrate that fructose administration in the face of impending hypoglycemia induces an earlier and more powerful counterregulatory response compared with control. Furthermore, the significant increase in glucose production during the 3.9- and 3.3-mmol/l glucose steps was present in all subjects, and our inability to lower the plasma glucose to the target glycemic level (3.3 mmol/l) in the fructose infusion studies was also noted in all subjects, factors that further strengthen our findings.

The fact that in the fructose infusion studies both epinephrine and glucagon responses were amplified suggests that fructose action occurred at the level of glucose sensing or at a more proximal integration center. Amplification of the glucagon response only, the epinephrine secretion only, or the EGP response alone could have suggested a local effect on the α -cell, autonomic activation, or CNS (or hepatic) regulation of EGP, respectively.

In conclusion, this study describes a novel finding that the counterregulatory hormonal response to hypoglycemia can be enhanced or reset in nondiabetic subjects with fructose. The augmentation of the counterregulatory response was characterized by a shift in the glycemic threshold for epinephrine and glucagon secretion (which occurred at a higher plasma glucose level) and an increased magnitude of these hormones. Augmenting the counterregulatory response to hypoglycemia could have major implications in the management of patients with type 1 diabetes in whom intensive insulin treatment, although advantageous, is often avoided because of the

risk of developing defective counterregulation and severe hypoglycemia. However, whether fructose has the same effects on hypoglycemia counterregulation in patients with type 1 diabetes remains to be determined.

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