

Inclusion of Low Amounts of Fructose With an Intraduodenal Glucose Load Markedly Reduces Postprandial Hyperglycemia and Hyperinsulinemia in the Conscious Dog

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Intraportal infusion of small amounts of fructose markedly augmented net hepatic glucose uptake (NHGU) during hyperglycemic hyperinsulinemia in conscious dogs. In this study, we examined whether the inclusion of catalytic amounts of fructose with a glucose load reduces postprandial hyperglycemia and the pancreatic β -cell response to a glucose load in conscious 42-h-fasted dogs. Each study consisted of an equilibration (-140 to -40 min), control (-40 to 0 min), and test period (0–240 min). During the latter period, glucose ($44.4 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) was continuously given intraduodenally with ($2.22 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) or without fructose. The glucose appearance rate in portal vein blood was not significantly different with or without the inclusion of fructose (41.3 ± 2.7 vs. $37.3 \pm 8.3 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, respectively). In response to glucose infusion without the inclusion of fructose, the net hepatic glucose balance switched from output to uptake (from 10 ± 2 to $11 \pm 4 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) by 30 min and averaged $17 \pm 6 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$. The fractional extraction of glucose by the liver during the infusion period was $7 \pm 2\%$. Net glycogen deposition was 2.44 mmol glucose equivalent/kg body wt; 49% of deposited glycogen was synthesized via the direct pathway. Net hepatic lactate production was 1.4 mmol/kg body wt. Arterial blood glucose rose from 4.1 ± 0.2 to 7.3 ± 0.4 mmol/l, and arterial plasma insulin rose from 42 ± 6 to 258 ± 66 pmol/l at 30 min, after which they decreased to 7.0 ± 0.5 mmol/l and 198 ± 66 pmol/l, respectively. Arterial plasma glucagon decreased from 54 ± 7 to 32 ± 3 ng/l. In response to intraduodenal glucose infusion in the presence of fructose, net hepatic glucose balance switched from $9 \pm 1 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ output to 12 ± 3 and $28 \pm 5 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ uptake by 15 and 30 min, respectively. The average NHGU ($28 \pm 5 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) and fractional extraction during infusion period

($12 \pm 2\%$), net glycogen deposition (3.68 mmol glucose equivalent/kg body wt), net hepatic lactate production (3.27 mmol/kg), and glycogen synthesis via the direct pathway (68%) were significantly higher ($P < 0.05$) compared to that in the absence of fructose. The increases in arterial blood glucose (from 4.4 ± 0.1 to 6.4 ± 0.2 mmol/l at 30 min) and arterial plasma insulin (from 48 ± 6 to 126 ± 30 pmol/l at 30 min) were significantly smaller ($P < 0.05$). In summary, the inclusion of small amounts of fructose with a glucose load augmented NHGU, increased hepatic glycogen synthesis via the direct pathway, and augmented hepatic glycolysis. As a result, postprandial hyperglycemia and insulin release by the pancreatic β -cell were reduced. In conclusion, catalytic amounts of fructose have the ability to improve glucose tolerance. *Diabetes* 51:469–478, 2002

Glucose uptake by the liver contributes in a major way to the disposal of alimentary glucose (1). In healthy humans, 20–30% of absorbed glucose is taken up by the liver, and hepatic glycogen synthesis accounts for the disposal of about 70% of that amount. Liver glycogen is synthesized by both direct (glucose \rightarrow glucose-6-phosphate [G6P] \rightarrow glucose-1-phosphate \rightarrow uridine 5'-diphosphate [UDP] glucose \rightarrow glycogen) and indirect (three carbon unit \rightarrow phosphoenolpyruvate \rightarrow G6P \rightarrow glucose-1-phosphate \rightarrow UDP glucose \rightarrow glycogen) pathways (2). After oral glucose ingestion in the healthy human, 50–77% of the liver glycogen synthesized is derived via the direct pathway (3–6). The response of the conscious dog is similar to that of humans, with 25–40% of a gastrointestinal glucose load being taken up by the liver and 50–62% of accumulated hepatic glycogen being synthesized via the direct pathway (7–9).

Individuals with diabetes exhibit excessive postprandial hyperglycemia, with a defect in meal- or glucose-induced suppression of endogenous glucose production (10–15). Only a few studies have examined the effect of type 2 diabetes on splanchnic glucose uptake, and the results from these data are not concordant. Several studies (12,13,15,16) have demonstrated that the greater net splanchnic glucose release observed in diabetic compared with nondiabetic subjects after glucose injection was attributable to excessive endogenous glucose production rather than to lower initial splanchnic extraction of the

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Received for publication 21 September 2001 and accepted in revised form 1 November 2001.

A.D.C. is on the Medical Advisory Board for Entelos, for which he receives a consulting fee and stock options.

APE, atom percent excess; CV, coefficient of variation; FIP, fructose-1-phosphate; F6P, fructose-6-phosphate; G6P, glucose-6-phosphate; NHGU, net hepatic glucose uptake; UDP, uridine 5'-diphosphate.

ingested glucose. However, the insulin and glucose concentrations differed in the diabetic and nondiabetic subjects in all of those studies, precluding direct comparison of the efficiency of splanchnic glucose uptake. DeFronzo et al. (17) and Ludvik et al. (18) compared the splanchnic glucose uptake during a hyperinsulinemic-euglycemic clamp in diabetic and nondiabetic subjects. Decreased splanchnic glucose uptake in diabetic subjects was found by Ludvik et al. (18), but not by DeFronzo et al. (17). However, it has been previously shown that in the presence of euglycemia, hyperinsulinemia only minimally stimulates splanchnic glucose uptake (17,19–21), whereas hyperglycemia combined with hyperinsulinemia substantially increases glucose uptake in the liver (19–22). Recently Basu et al. (23) carried out a hyperglycemic and hyperinsulinemic clamp study in human subjects and showed that the increase in splanchnic glucose uptake and the suppression of splanchnic glucose production were lower in type 2 diabetic subjects as compared to normal subjects. They also showed that the flux through the UDP-glucose pool and the contribution of the direct pathway to glycogen synthesis were also decreased in the diabetic subjects, indicating a decrease in hepatic uptake of extracellular glucose (19). A reduced rate of hepatic glycogen synthesis from glucose via the direct pathway has been reported by other studies (24–26). The same alterations in hepatic glucose metabolism have been found in various animal models of diabetes (21,27,31). GLUT2 expression is increased by high glucose concentrations (32). Because the presence of GLUT2 in the liver allows a rapid equilibration of the intracellular glucose level with the extracellular glucose level (33,34), net hepatic glucose flux represents a balance between glucokinase and glucose-6-phosphatase flux. Therefore it is likely that the excessive postprandial hyperglycemia evident in diabetic subjects is, in part, caused by a defect in net hepatic glucose uptake (NHGU) resulting from impaired glucose phosphorylation catalyzed by glucokinase and/or an increase in glucose dephosphorylation attributable to glucose-6-phosphatase.

Small amounts of fructose have been reported to activate glucokinase in a catalytic manner. Van Shaftingen et al. (35) demonstrated that glucokinase activity is acutely regulated by its interaction with a regulatory protein. The regulatory protein binds to glucokinase and allosterically inhibits it by decreasing the apparent affinity of the enzyme for glucose. The regulatory protein with fructose-6-phosphate (F6P) bound is in a conformation capable of interacting with, and inhibiting, glucokinase. Fructose-1-phosphate (F1P) competes with F6P for binding to the regulatory protein. The regulatory protein with F1P bound is in a conformation that is not capable of interacting with glucokinase; thus glucokinase is not inhibited. Van Shaftingen et al. (35) and Agius et al. (36) have shown that the addition of very low concentrations of fructose rapidly increases F1P content in the hepatocyte and induces the release of glucokinase from its regulatory protein. Indeed, recent studies have shown that fructose at low extracellular concentrations (50–200 $\mu\text{mol/l}$) stimulates glucose phosphorylation, as measured by the formation of $^3\text{H}_2\text{O}$ from $[2\text{-}^3\text{H}]\text{glucose}$, and glycolytic flux, as measured by the release of $^3\text{H}_2\text{O}$ from $[3\text{-}^3\text{H}]\text{glucose}$ (35,37,38). Recently we

have shown that intraportal infusion of a small amount of fructose at 1.7, 3.3, or 6.7 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, which raised the portal blood fructose concentration from <6 (basal) to 113, 209, and 426 $\mu\text{mol/l}$, respectively, increased NHGU from 15 to 41, 54, and 69 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, respectively, during a hyperglycemic, hyperinsulinemic clamp in 42-h-fasted dogs (39). The glucose that entered the liver was stored as glycogen (69%), released as lactate (17%), or oxidized (8%). Almost all (90%) of the stored glycogen was deposited via the direct pathway. These observations suggest that very low amounts of fructose might be able to stimulate NHGU and glycogen synthesis resulting from an oral glucose load via the activation of glucokinase in intact animals.

To evaluate whether a catalytic amount of fructose given orally can lessen postprandial hyperglycemia and thereby reduce the demand on β -cells, we examined the effects of including small amounts of fructose with an intraduodenal glucose load on the resulting increments of plasma glucose and insulin in conscious dogs.

RESEARCH DESIGN AND METHODS

Animals and surgical procedures. Experiments were performed on 18 42-h-fasted mongrel dogs (~ 18.7 to ~ 27.8 kg; mean 23.5 ± 0.8 kg) of either sex, which had been fed a standard meat and chow diet (34% protein, 46% carbohydrate, 14% fat, and 6% fiber based on dry weight; Kal Kan, Vernon, CA; Purina Lab Canine Diet No. 5006, Purina Mills, St. Louis, MO) once daily. The dogs were housed in a facility that met American Association for the Accreditation of Laboratory Animal Care guidelines, and the protocols were approved by the Vanderbilt University Medical Center Animal Care Committee. At least 16 days before an experiment, a laparotomy was performed under general endotracheal anesthesia (15 mg/kg pentothal sodium before surgery and 0.1% isoflurane as an inhalation anesthetic during surgery), and catheters for blood sampling were placed into a femoral artery, the portal vein, and a hepatic vein, as previously described (7,9,39). An additional catheter was inserted into the duodenum through a purse-string suture 3.3–4.0 cm below the pylorus. The distal tip was positioned ~ 3 cm inside the bowel. Transonic flow probes were placed on the hepatic artery and portal vein. On the day of the experiment, the catheters were exteriorized under local anesthesia (2% lidocaine; Abbott, North Chicago, IL), their contents were aspirated, and they were flushed with saline.

On the day before the experiment, the leukocyte count and hematocrit were determined. Dogs were used for an experiment only if they had a leukocyte count $<18,000/\text{mm}^3$, a hematocrit $>38\%$, a good appetite, and normal stools.

Experimental design. After a 100-min (-140 to -40 min) equilibration period, there was a 40-min (-40 to 0 min) control period and then a 240-min (0 – 240 min) test period. During the test period, a glucose infusate consisting of 20% dextrose (20% enriched with $[1\text{-}^{13}\text{C}]\text{glucose}$) was administered with and without fructose via the duodenostomy tube. At $t = 0$, a bolus of infusate sufficient to provide 840 μmol glucose/kg was given with (test group, $n = 9$) or without (control group, $n = 9$) 42 μmol fructose/kg, and a constant infusion providing 44.2 μmol glucose $\cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ was started with or without 2.22 μmol fructose $\cdot \text{kg}^{-1} \cdot \text{min}^{-1}$.

Analytical procedures. Plasma glucose concentrations were determined using the glucose oxidase method in a Beckman glucose analyzer (Beckman Instruments, Fullerton, CA) (40). Blood concentrations of lactate, glycerol, alanine, and 3-hydroxybutyric acid were determined according to the method of Lloyd et al. (41) adapted to the Monarch 2000 centrifugal analyzer (Lexington, MA) in samples deproteinized with perchloric acid. Blood fructose concentrations were determined as previously reported (39).

Liver samples were obtained at the end of experiments by anesthetizing the dog with pentobarbital sodium, exposing the liver by laparotomy, and freeze clamping the liver in situ in <2 min. The entire liver was then removed from the dog and weighed. The frozen samples were stored at -70°C for subsequent analysis. On the day of the assay, samples were powdered and the glycogen was extracted and purified as previously described (42). The cold glycogen concentrations were determined by acid hydrolysis and enzyme degradation using α -1,4,6-amyloglucosidase (43). The ^{13}C enrichment patterns of the glucosyl residues within the glycogen were determined by nuclear magnetic resonance, as previously described (42).

Immunoreactive plasma insulin was measured using a double-antibody procedure (interassay coefficient of variation [CV] of 8%) (44). Immunoreactive glucagon concentrations were measured in plasma containing 500 KIU/ml aprotinin (Trasylol; FBA Pharmaceuticals, New York, NY) by a double-antibody radioimmunoassay, using a method similar to that used in the insulin assay, with a CV of 9% (45). Plasma cortisol was measured with the Clinical Assay Gamma Coat Radioimmunoassay kit (CV of 8%; Clinical Assays, Travenol-Genentech Diagnostics, Cambridge, MA). Plasma epinephrine and norepinephrine were determined by high-performance liquid chromatography, as previously described (46), with CVs of 10 and 5%, respectively.

Calculations. The hepatic arterial and portal blood flow were measured by transonic flow probes. The net hepatic substrate balance was calculated using the formula $[H(Fa + Fp) - AFa - PFp]$, where H, A, and P are the hepatic vein, arterial, and portal vein substrate concentrations, respectively, and Fa and Fp are hepatic, arterial, and hepatic portal vein blood or plasma flows, respectively. Net fractional substrate extraction by the liver was calculated as the ratio of net hepatic balance to hepatic load. Net gut balance was determined by multiplying the arterial-portal substrate difference by the portal blood flow.

The percentage of glycogen synthesized by the direct pathway was determined by the following equation:

$$\text{Percentage of direct pathway} = (C1 \text{ glycogen} - C6 \text{ glycogen}) \cdot 100 / C1pv \cdot CF$$

where C1 and C6 glycogen represent the ^{13}C atom percent excess (APE) in positions 1 and 6, respectively, of the glucosyl residues from hepatic glycogen; C1pv represents the APE in carbon 1 of portal vein plasma glucose; and CF is a correction factor to account for the amount of time required for portal vein ^{13}C to reach steady state. A CF value was determined for each dog based on plasma samples obtained every 10–30 min throughout the glucose infusion period and analyzed by gas chromatography–mass spectrometry. Thus the expression “C1pv · CF” represents the enrichment in the C1 position of the portal vein plasma glucose, corrected by the integrated area under the curve of APE plotted against time. The use of this factor assumes that hepatic glycogen synthesis proceeds in a linear fashion, as has been previously shown in rats (27). The calculation was modified to compensate for the mass of glycogen present in the liver and for the natural abundance of ^{13}C in hepatic glycogen at the beginning of the glucose infusion. Thus, the modified equation was as follows:

$$\left[\frac{(C1 \cdot \text{Glyf}) - (1.1 \cdot 2.52)}{\text{Glyf} - 2.52} \right] - \left[\frac{(C6 \cdot \text{Glyf}) - (1.1 \cdot 2.52)}{\text{Glyf} - 2.52} \right] \\ \times \left[\frac{1 \times 100}{C1pv \times CF} \right]$$

where Glyf is the glycogen concentration at the end of the glucose infusion (g/100 g liver), $1.1 \pm 0.5\%$ is the natural abundance of ^{13}C in glycogen from control animals that did not receive the glucose infusion (and thus the projected natural abundance in the experimental animals at the beginning of the glucose infusion), and 2.52 ± 0.08 g/100 g liver is the glycogen concentration in the control animals (and the projected concentration in the experimental animals at the beginning of the glucose infusion).

Statistical analysis. Data are expressed as means \pm SE. A one-way ANOVA for repeated measures was used to analyze changes over time, and a two-way ANOVA for repeated measures was used to compare time course differences between groups. When significant changes were obtained over time, post hoc comparisons were made using a paired *t* test. $P < 0.05$ was considered statistically significant.

RESULTS

Hepatic blood flow. The hepatic portal venous and arterial blood flows in the control and experimental periods were similar in the two groups (Fig. 1). The hepatic portal blood flow rose by $\sim 10\%$ immediately after the glucose infusion was started, but it returned to the basal rate within 1–2 h. The hepatic arterial blood flow did not change during glucose infusion and remained constant throughout the experiment.

Blood fructose concentration, gut and hepatic fructose balance, and hepatic fructose fractional extraction. Basal fructose levels in arterial and portal blood were similar in both the control (9 ± 3 and 10 ± 3 $\mu\text{mol/l}$, respectively) and the test groups (12 ± 4 and 14 ± 4

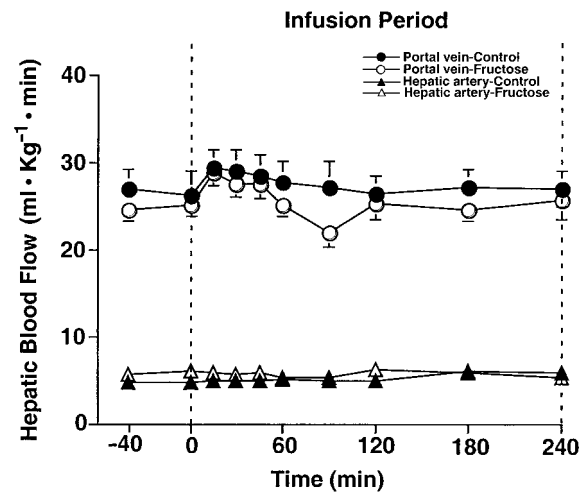


FIG. 1. Hepatic artery and portal vein blood flow during control period (–40 to 0 min) and continuous intraduodenal infusion of glucose at $44.4 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ with and without continuous intraduodenal infusion of fructose at $2.22 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ in 42-h-fasted conscious dogs. Data represent means \pm SE; $n = 9$ for each group.

$\mu\text{mol/l}$, respectively) (Fig. 2). The intraduodenal infusion of glucose alone did not alter the fructose concentrations in arterial (10 ± 2 $\mu\text{mol/l}$) or hepatic portal venous blood (10 ± 3 $\mu\text{mol/l}$). The intraduodenal fructose infusion at $2.22 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ increased arterial and portal concentrations of the sugar to 28 ± 4 and 86 ± 15 $\mu\text{mol/l}$, respectively, by 10 min, after which they averaged 38 ± 5 and 100 ± 14 $\mu\text{mol/l}$, respectively. The mean rate of net fructose absorption during the infusion period was $1.62 \pm 0.28 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, which was equivalent to 73% of its infusion rate. The average rate of net hepatic fructose uptake was $1.36 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, which was equivalent to $\sim 84\%$ of the absorption rate by the gut. The average net hepatic fractional extraction of fructose during the infusion period was 46%.

Plasma glucose concentrations, gut and hepatic glucose balance, hepatic glucose balance, and fractional extraction. In response to intraduodenal glucose infusion alone, the arterial plasma glucose level rose from 5.6 ± 0.2 to 10.2 ± 0.4 mmol/l by 30 min and then averaged 9.5 ± 0.5 mmol/l; the portal vein plasma glucose level rose from 5.4 ± 0.2 to 12.2 ± 0.7 mmol/l by 30 min and then averaged 11.5 ± 0.6 mmol/l (Fig. 3). In the presence of intraduodenal fructose infusion, the arterial plasma glucose level rose from 6.0 ± 0.1 to 8.8 ± 0.3 mmol/l by 30 min and then gradually fell to 7.3 ± 0.2 mmol/l; the portal vein plasma glucose level rose from 5.9 ± 0.1 to 10.8 ± 0.4 mmol/l by 30 min, after which it gradually fell to 9.8 ± 0.3 mmol/l by 240 min. The increment in the arterial plasma glucose level in the presence of fructose infusion (2.7 ± 0.4 at 30 min [$P < 0.05$] and 1.2 ± 0.1 at 240 min [$P < 0.05$]) was about half that of the control group in the absence of fructose infusion (4.6 ± 0.3 at 30 min and 4.1 ± 0.5 at 240 min). On the other hand, the arterial-portal difference in plasma glucose was similar in the presence (2.33 ± 0.1) and absence (2.27 ± 0.2 mmol/l) of fructose infusion.

Net gut glucose absorption did not differ significantly in the presence or absence of fructose infusion (41.3 ± 2.7 vs. $37.3 \pm 8.3 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, respectively) (Fig. 4). Net glucose uptake by the gut before the glucose infusion was

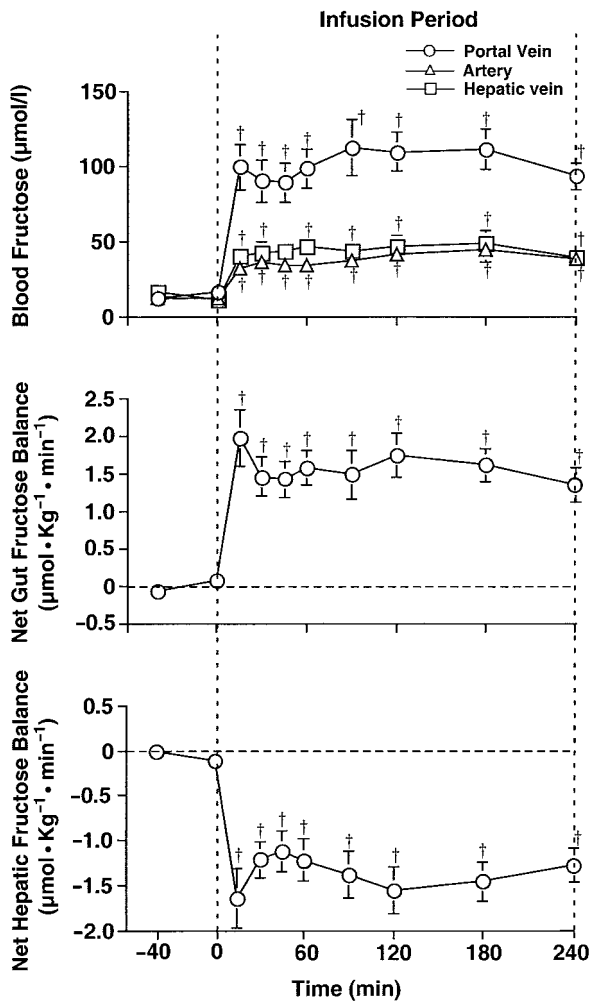


FIG. 2. Arterial, portal, and hepatic vein fructose levels and changes in net hepatic fructose balance before and during continuous intraduodenal infusion of glucose at $44.4 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ with continuous intraduodenal infusion of fructose at $2.22 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ in 42-h-fasted conscious dogs. Data represent means \pm SE; $n = 9$ for each group. $\dagger P < 0.05$ vs. control period in identical vessel.

2.6 ± 0.6 and $5.3 \pm 1.1 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ in the presence and absence of fructose infusion, respectively. If we assume that gut glucose consumption persisted during glucose infusion, we can account for $99 \pm 5\%$ and $96 \pm 11\%$ of the administrated glucose in the presence and absence of fructose infusion, respectively.

Before the start of the infusion, net hepatic glucose outputs were similar in the presence and absence of fructose infusion (9.3 ± 0.7 vs. $9.9 \pm 1.7 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, respectively) (Fig. 5). In the control group, net hepatic glucose production was completely shut down by 15 min ($-0.7 \pm 2.0 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$), after which the liver switched from output to uptake, reaching steady state ($17.3 \pm 5.6 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) by 45 min. In the presence of fructose infusion, on the other hand, net hepatic glucose balance switched to uptake ($11.6 \pm 3.3 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) at 15 min and reached steady state ($28.3 \pm 4.6 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) at 30 min. Net hepatic fractional extraction of glucose was 4% at 30 min and reached 9% at 240 min in the absence of fructose infusion, but was doubled (11%) in the presence of fructose infusion at 30 min and reached 15% at 240 min.

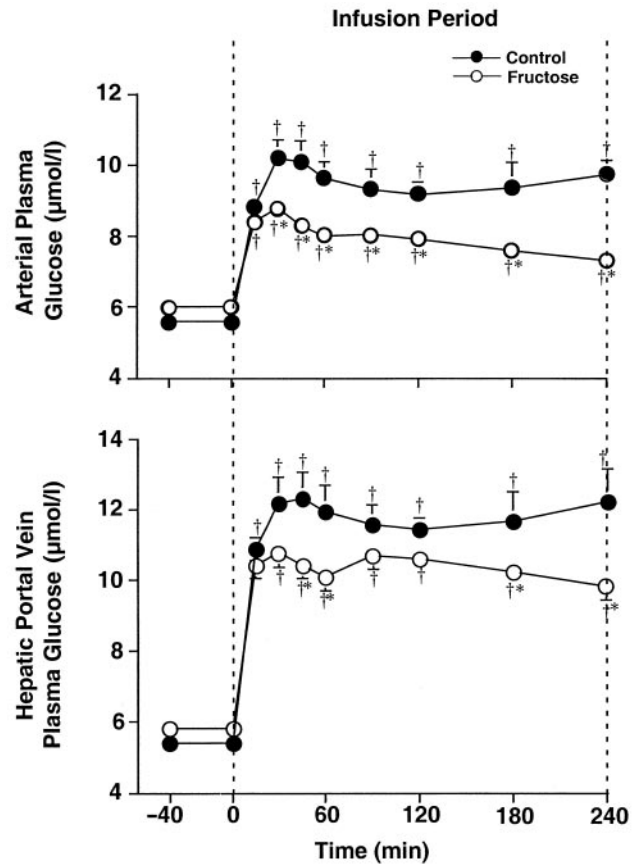


FIG. 3. Arterial and portal vein plasma glucose levels before and during continuous intraduodenal infusion of glucose at $44.4 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ with and without continuous intraduodenal infusion of fructose at $2.22 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ in 42-h-fasted conscious dogs. Data represent means \pm SE; $n = 9$ for each group. $\dagger P < 0.05$ vs. control period in identical group; $P < 0.05$ vs. corresponding value in control group.

Lactate concentration and metabolism. Arterial blood lactate concentrations and net hepatic lactate uptakes in the control period were similar in the two groups (Fig. 6). In response to the intraduodenal glucose infusion, the liver

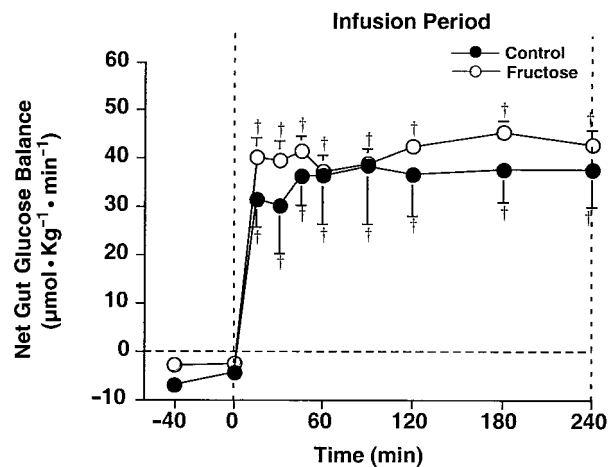


FIG. 4. Net gut glucose balance before and during continuous intraduodenal infusion of glucose at $44.4 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ with and without continuous intraduodenal infusion of fructose at $2.22 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ in 42-h-fasted conscious dogs. Data represent mean \pm SE; $n = 9$ for each group. $\dagger P < 0.05$ vs. control period in identical group.

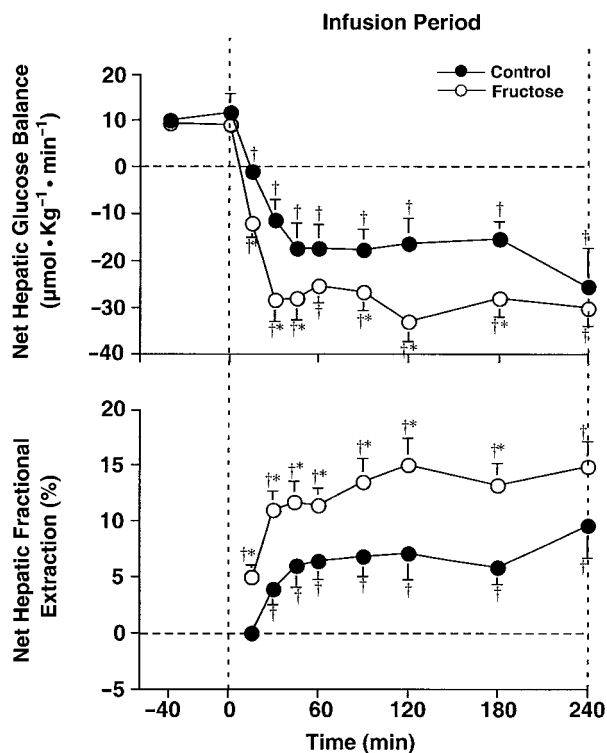


FIG. 5. Net hepatic glucose balance and hepatic fractional glucose extraction before and during continuous intraduodenal infusion of glucose at $44.4 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ with and without continuous intraduodenal infusion of fructose at $2.22 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ in 42-h-fasted conscious dogs. Data represent means \pm SE; $n = 9$ for each group. $\dagger P < 0.05$ vs. control period in identical group; $*P < 0.05$ vs. corresponding value in control group.

switched from net uptake to net output of lactate in both groups. The area under the curve for output was twofold greater in the presence of fructose.

Alanine concentration and metabolism. The arterial blood alanine concentration rose slightly during the intraduodenal infusion of glucose alone and during combined intraduodenal glucose and fructose infusion (Table 1). Net hepatic alanine uptake rose slightly in both groups, but the hepatic fractional extraction of alanine did not change significantly in either group. Cumulative net hepatic alanine uptakes during the glucose infusion period were $760 \pm 82 \mu\text{mol}/\text{kg}$ in the presence and $759 \pm 52 \mu\text{mol}/\text{kg}$ in the absence of the fructose infusion.

Glycerol concentration and metabolism. In response to intraduodenal glucose given in the presence and absence of fructose infusion, the arterial blood glycerol concentration and net hepatic glycerol uptake decreased. The cumulative net hepatic glycerol uptakes during the glucose infusion period were $144 \pm 32 \mu\text{mol}/\text{kg}$ in the presence and $288 \pm 42 \mu\text{mol}/\text{kg}$ in the absence of the fructose infusion.

Hepatic disposition of glucose and gluconeogenic precursors. Previously we showed that the hepatic glycogen content of the 42-h-fasted dog was $139 \pm 6 \mu\text{mol}$ glucose equivalents/g liver tissue. In the control group, the hepatic glycogen content was $249 \pm 21 \mu\text{mol}$ glucose equivalents/g at the end of the experiment. Glycogen synthesis via the direct pathway accounted for $49 \pm 9\%$ of total glycogen deposition. In the fructose group, on the other hand, the hepatic glycogen content was 321 ± 32

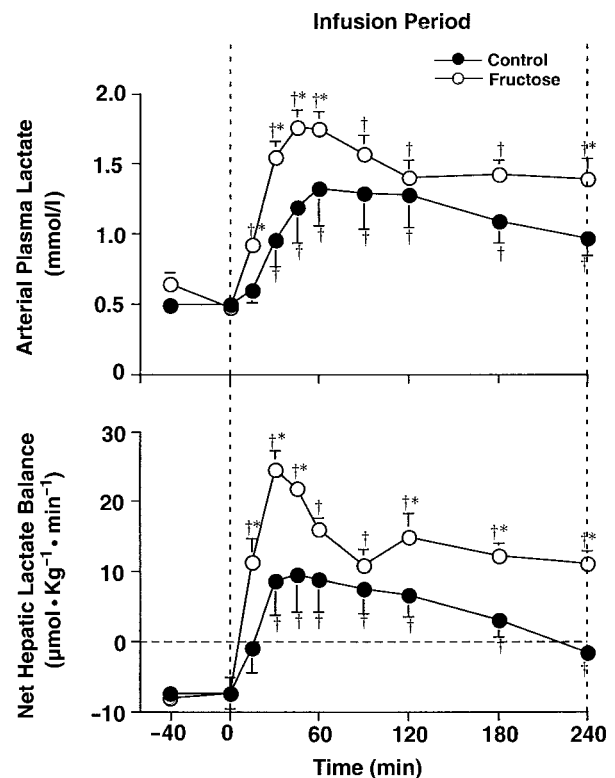


FIG. 6. Arterial blood lactate levels and net hepatic lactate balance before and during continuous intraduodenal infusion of glucose at $44.4 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ with and without continuous intraduodenal infusion of fructose at $2.22 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ in 42-h-fasted conscious dogs. Data represent means \pm SE; $n = 9$ for each group. $\dagger P < 0.05$ vs. control period in identical group; $*P < 0.05$ vs. corresponding value in control group.

μmol glucose equivalents/g liver tissue at the end of experiment, and glycogen synthesis via the direct pathway contributed $68 \pm 8\%$ of the new glycogen formed.

Hormonal concentrations. In response to intraduodenal infusion of glucose alone, the arterial insulin level rose from 42 ± 6 at basal to $258 \pm 66 \text{ pmol}/\text{l}$ at 30 min, then plateaued at $198 \pm 66 \text{ pmol}/\text{l}$ (Fig. 7). The arterial plasma glucagon level declined from 54 ± 7 to $32 \pm 3 \text{ ng}/\text{l}$ during the glucose infusion period. When glucose was infused with fructose, the arterial insulin level rose from 48 ± 6 during the control period to $126 \pm 30 \text{ pmol}/\text{l}$ at 30 min, after which it declined to $96 \pm 24 \text{ pmol}/\text{l}$ by the end of the infusion period. The arterial plasma glucagon level did not change significantly during the infusion period.

DISCUSSION

The results of the present study demonstrate that in the dog, small amounts of fructose can significantly lessen the hyperglycemia and hyperinsulinemia resulting from intraduodenal glucose infusion. Further they show that this improvement results from a marked increase in the ability of the liver to take up glucose and store glycogen.

Intestinal fructose absorption and fructose metabolism. When fructose was infused at 1.7, 3.3, and $6.7 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ into the portal vein, our techniques could accurately measure the appearance of fructose in the portal blood (91, 81, and 93%, respectively, of the infused hexose was recovered) (39). On the other hand, when a small amount of fructose was infused into the duodenum,

TABLE 1

Arterial blood levels, net hepatic uptake, and net hepatic fractional extractions of alanine and glycerol before and during continuous intraduodenal infusion of glucose ($44.4 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) with and without continuous intraduodenal infusion of fructose ($2.22 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) in 42-h-fasted conscious dogs

Group	Control period	Infusion period (min)					
		30	60	90	120	180	240
Alanine							
Control							
Arterial concentration ($\mu\text{mmol/l}$)	264 ± 23	286 ± 20	319 ± 29	$339 \pm 27^*$	$348 \pm 23^*$	$358 \pm 33^*$	$333 \pm 45^*$
Net hepatic uptake ($\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)	2.78 ± 0.33	2.84 ± 0.41	2.84 ± 0.40	3.49 ± 0.33	3.40 ± 0.26	4.11 ± 0.37	3.33 ± 0.37
Net fractional extraction	0.30 ± 0.04	0.25 ± 0.04	0.23 ± 0.03	0.28 ± 0.03	0.28 ± 0.02	0.31 ± 0.02	0.28 ± 0.03
Fructose							
Arterial concentration ($\mu\text{mmol/l}$)	366 ± 27	428 ± 30	$486 \pm 33^*$	$510 \pm 35^*$	$488 \pm 33^*$	$523 \pm 39^*$	$511 \pm 38^*$
Net hepatic uptake ($\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)	2.78 ± 0.19	2.92 ± 0.27	3.06 ± 0.30	2.94 ± 0.31	3.54 ± 0.40	$3.77 \pm 0.33^*$	$3.61 \pm 0.27^*$
Net fractional extraction	0.26 ± 0.04	0.20 ± 0.02	0.21 ± 0.03	0.22 ± 0.03	0.24 ± 0.04	0.25 ± 0.04	0.24 ± 0.03
Glycerol							
Control							
Arterial concentration ($\mu\text{mmol/l}$)	126 ± 16	87 ± 13	$79 \pm 15^*$	$70 \pm 14^*$	$71 \pm 19^*$	$57 \pm 13^*$	$52 \pm 5^*$
Net hepatic uptake ($\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)	2.19 ± 0.33	1.72 ± 0.21	1.91 ± 0.34	$1.22 \pm 0.24^*$	$0.90 \pm 0.17^*$	$1.01 \pm 0.37^*$	$1.10 \pm 0.24^*$
Net fractional extraction	0.55 ± 0.06	0.60 ± 0.09	0.68 ± 0.08	0.53 ± 0.13	0.46 ± 0.09	0.44 ± 0.12	0.61 ± 0.13
Fructose							
Arterial concentration ($\mu\text{mmol/l}$)	81 ± 8	$39 \pm 6^*$	$35 \pm 8^*$	$27 \pm 2^*$	$32 \pm 7^*$	$41 \pm 9^*$	$37 \pm 4^*$
Net hepatic uptake ($\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)	1.62 ± 0.20	$0.63 \pm 0.18^*$	$0.54 \pm 0.14^*$	$0.48 \pm 0.17^*$	$0.59 \pm 0.11^*$	$0.59 \pm 0.14^*$	$0.59 \pm 0.12^*$
Net fractional extraction	0.61 ± 0.03	0.43 ± 0.05	0.56 ± 0.04	0.44 ± 0.07	0.55 ± 0.03	0.47 ± 0.04	0.51 ± 0.06

Data are means \pm SE; $n = 9$ dogs for each group. *Significantly changed from the values during control period in same group.

the appearance rate ($1.62 \pm 0.28 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) of fructose in portal vein blood could account for only 73% of the infusion rate ($2.22 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) of the sugar. The intestine, as well as the liver and the kidney, possesses the enzymes fructokinase, aldolase B, and triokinase that catalyze fructose metabolism (47). In experiments in which fructose was infused through a peripheral vein in 42-h-fasted dogs, net gut fructose uptake accounted for $\sim 10\%$ of the fructose reaching the gut (39). It is likely, therefore, that our failure to observe complete recovery of the infused fructose was, in large part, explained by the metabolism of fructose by the gut.

In humans, $\sim 50\%$ of the fructose given by prolonged intravenous infusion is taken up by the splanchnic tissues, with the liver accounting for 75% of this removal (47,48). In previous experiments in which fructose was infused into the portal vein at a range of 1.7 to $\sim 6.7 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ in the presence of hyperglycemic hyperinsulinemia in conscious dogs (39), the hepatic fractional extraction (first pass extraction) of fructose was 50% over a wide range of sinusoidal fructose concentrations (60 to $\sim 300 \mu\text{mol/l}$) and net hepatic fructose uptake could account for $\sim 77\%$ of the fructose infused into the portal vein. In the present study in which the hepatic sinusoidal fructose concentrations were $90 \mu\text{mol/l}$ during the intraduodenal infusion of the sugar at $2.22 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, the hepatic fractional extraction of fructose was 46% and the net hepatic fructose uptake could account for 84% of the fructose absorbed. These results confirm that the liver is the major site of fructose clearance in vivo.

Fructose-induced reduction of postprandial hyperglycemia. During the delivery of glucose without fructose, the hepatic fractional extraction of glucose averaged $\sim 6\%$;

at steady state, the hepatic glucose uptake accounted for $\sim 48\%$ of the absorption rate of the sugar. These results were consistent with previously reported values obtained under similar conditions (7,9). With the inclusion of fructose, NHGU increased by 50%, with a marked increase in the fractional extraction of glucose by the liver (13%). In the presence of fructose, the liver took up glucose at a rate equal to 70% of the glucose absorption rate. The arterial-portal difference in the plasma glucose concentration was similar in both groups (Fig. 3), indicating that a difference in the magnitude of the portal signal could not explain the results. The plasma glucose and insulin levels increased less in the presence of fructose than in its absence, thereby opposing increased hepatic glucose uptake. On the other hand, the sinusoidal fructose concentration rose from 13 ± 4 (basal) to $88 \pm 11 \mu\text{mol/l}$ in response to the infusion of fructose. When the sinusoidal fructose concentration was raised from 6 ± 2 to $98 \pm 10 \mu\text{mol/l}$ by intraportal infusion of the sugar in the presence of hyperinsulinemic hyperglycemia in a previous study, NHGU was markedly increased (from 14 ± 2 to $41 \pm 3 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) (39). We concluded, therefore, that the increased NHGU caused by the inclusion of fructose was the direct effect of the ketohexose on the liver.

Net extrahepatic glucose uptake, which was calculated from the difference between the appearance rate of glucose in the portal blood and the NHGU rate, was lower in the presence of fructose infusion. Because intraportal infusion of a small amount of fructose did not affect extrahepatic glucose clearance during hyperglycemic hyperinsulinemia in our previous study (39), decreased glucose disposal in the extrahepatic tissues was probably secondary to the decreased plasma glucose and insulin

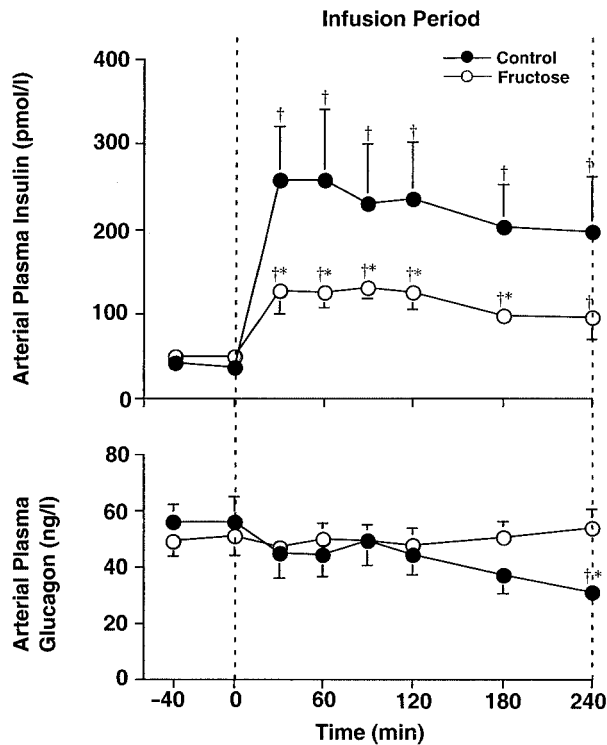


FIG. 7. Arterial plasma levels of insulin and glucagon before and during continuous intraduodenal infusion of glucose at $44.4 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ with and without continuous intraduodenal infusion of fructose at $2.22 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ 42-h-fasted conscious dogs. Data represent means \pm SE; $n = 9$ for each group. † $P < 0.05$ vs. control period in identical group; * $P < 0.05$ vs. corresponding value in control group.

levels that occurred when glucose and fructose were given together. It is clear, therefore, that the decrease in the increment of plasma glucose caused by catalytic amounts of fructose was attributable to an increased efficiency of NHGU.

Recently Dirlewanger et al. (49) reported that in humans, the intravenous (peripheral) infusion of fructose at $16.7 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ caused insulin resistance in the extrahepatic tissue(s) as well as in the liver. In the present study, in contrast, the intraduodenal fructose infusion ($2.22 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) did not affect glucose disposal in the extrahepatic tissue(s). The appearance rate of fructose in the portal vein during the intraduodenal infusion of fructose was $1.62 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ (Fig. 2), which is only 10% of the fructose infusion rate used by Dirlewanger et al. (49). Furthermore, because 84% of the absorbed fructose was taken up by the liver during the first pass (Fig. 2), only 16% of the absorbed fructose ($0.26 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) reached the systemic circulation. It would be expected that the arterial concentrations of fructose during fructose infusion would be >50 times higher in Dirlewanger et al. (49) than in the present study, although Dirlewanger et al. did not show the arterial concentration of fructose. Therefore, the difference in the effect of fructose infusion on the responsiveness of the extrahepatic tissue(s) to insulin between Dirlewanger et al.'s and our study may be attributable to the difference in the fructose concentrations in the arterial blood.

Insulin secretion. The increment in plasma insulin levels seen in response to the intraduodenal glucose load was markedly less in the presence than in the absence of

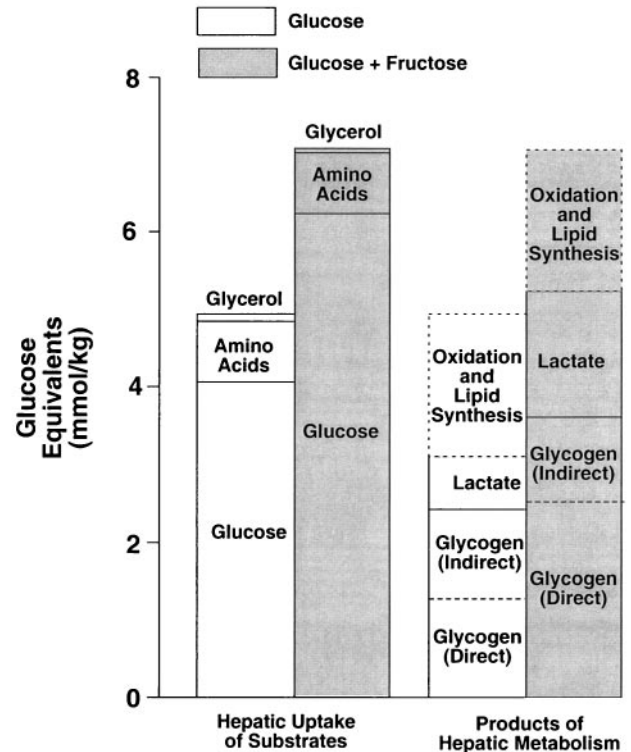


FIG. 8. Cumulative hepatic balance during continuous intraduodenal infusion of glucose at $44.4 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ with and without continuous intraduodenal infusion of fructose at $2.22 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ 42-h-fasted conscious dogs. $n = 9$ for each group.

fructose (Fig. 7). Fructose can be phosphorylated by glucokinase and fructokinase in the islets (50–53). It has been reported that glucokinase activity in islet homogenates is slightly increased by F1P (54,55) and that islet homogenates inhibit rat liver glucokinase, but not in the presence of F1P (54). β -cell glucokinase can be inhibited by the liver regulatory protein (56). It has been reported that a large amount of fructose (10–30 mmol/l) could increase insulin secretion in the presence of D-glucose (50), mannose (57), and arginine (58), and decrease arginine-induced glucagon secretion (58). However D-fructose (10 mmol/l) failed to affect insulin output from the islet in the absence of D-glucose (50) in perfused pancreas isolated from rats. Furthermore, it is unclear whether the low concentrations of fructose ($\sim 40 \mu\text{mol/l}$) seen during the fructose infusion in the present study can affect insulin secretion. Nevertheless, the potential stimulatory effects of fructose on insulin secretion would oppose the decrease in insulin secretion seen in response to the addition of fructose to intraduodenal glucose infusion. The lower increment of plasma insulin in response to the intraduodenal glucose infusion in the presence of fructose must therefore be secondary to the lower increment in plasma glucose.

Hepatic glycogen synthesis, glycolysis, and gluconeogenesis. As shown in Fig. 8, during intraduodenal glucose infusion without the inclusion of fructose, the cumulative net glucose uptake by the liver for the 240-min infusion period was 4.06 mmol/kg . In a net sense, the liver also took up 0.76 mmol glucose equivalent (C6)/kg of gluconeogenic amino acids and 0.15 mmol C6/kg of glycerol. Net glycogen deposition in the liver was 2.44 mmol C6/kg, and in a net

sense the liver produced 0.69 mmol C6/kg of lactate; 49% of the deposited glycogen (1.12 mmol C6/kg) was synthesized via the direct pathway. Therefore, the rest of the deposited glycogen (1.24 mmol C6/kg) was synthesized via the indirect pathway. The inclusion of fructose with glucose increased the cumulative NHGU by 50% (6.22 mmol C6/kg), but did not affect the cumulative net amino acid (0.79 mmol C6/kg) or glycerol (0.08 mmol C6/kg) uptake. The net glycogen deposition (3.68 mmol C6/kg) in the liver was also increased by 50%, with an increase in the contribution of the direct pathway for glycogen synthesis (68%). As a result, the amount of glycogen synthesized via the direct pathway (2.5 mmol C6/kg) was doubled, whereas that via the indirect pathway (1.18 mmol C6/kg) was not changed by the inclusion of fructose. Net lactate production (1.64 mmol C6/kg) by the liver doubled with the inclusion of fructose. Therefore, the inclusion of a small amount of fructose increased glycolysis and glycogen synthesis via the direct pathway.

Net glycogen deposition depends on the activities of glycogen synthase and phosphorylase. The effects of fructose administration on phosphorylase activity are controversial (45,53,57,58). Gergely et al. (59) and Bollen et al. (60) observed an activation of phosphorylase and an inhibition of phosphorylase phosphatase by F1P in liver extracts. In contrast, Kaufmann and Froesch (61) showed an inhibition of phosphorylase by F1P. On the other hand, an activation of hepatic glycogen synthase has been repeatedly observed with fructose administration during *in vivo* and *in vitro* studies (48). Several studies (62,63) have suggested that activation of glycogen synthase by the administration of relatively small fructose loads to intact animals is secondary to increased G6P, a potent activator for glycogen synthase (64). In our previous study in 42-h-fasted conscious dogs (39), an increase in NHGU resulting from an intraportal infusion of a small amount of fructose was accompanied by an increase in hepatic G6P content. It is possible, therefore, that fructose activates glycogen synthase via an increase in the intracellular content of G6P, which in turn results from the translocation of glucokinase and an increase hepatic glucose uptake.

The inclusion of a small amount of fructose with the intraduodenal glucose load increased net hepatic lactate production, indicating that fructose stimulated glycolysis in the liver. The small amount of fructose infused probably caused an increase in G6P content in the liver (39), and the increased content of this metabolite in cell likely increased glycolytic flux by mass action. In addition, it is known that pyruvate kinase and phosphofructokinase are key regulatory sites in glycolysis. When given to intact animals (65) or isolated hepatocytes (37), low fructose loads were shown to increase the intracellular content of fructose-2,6-diphosphate, a potent activator of phosphofructokinase (66). This effect might be secondary to increased G6P content, as fructose-induced activation of glycolysis was not observed in rat hepatocytes incubated in the presence of mannoheptulose, which inhibited glucokinase (37). Pyruvate kinase in the perfused liver has been reported to be activated by only very high fructose loads (1–5 mmol/l) (67). Therefore, the increased glycolytic flux associated with the inclusion of fructose in the present study is

probably secondary to an increase in the intracellular G6P content, which in turn is secondary to the activation of glucokinase, perhaps with an associated increase in the activity of phosphofructokinase.

Phosphorylation of fructose in the liver is not catalyzed by glucokinase, and fructose metabolism is not regulated directly by insulin (47). When normal and diabetic subjects consumed fructose, the postprandial increases in plasma glucose levels were less than those produced by isocaloric amounts of dextrose or sucrose (68–70). For this reason, the use of fructose as a substitute for dietary sucrose or dextrose has been examined as a potential therapeutic intervention for individuals with diabetes. However, in studies in which the effects of fructose on glucose metabolism were examined after several days or weeks of carbohydrate feeding (47,71,72), it has been shown that the conversion of glucose to glycogen, glucose oxidation, and lipid synthesis in the liver, muscle, and adipose tissues decreased. These adverse effects mean that substitution of fructose for glucose in the diet carries a significant risk (71). The difference in the amount of fructose used in the above studies versus the present experiments must be stressed. In our previous study, we showed that intraportal infusion of small amounts of fructose augmented NHGU markedly in the presence of hyperglycemia and hyperinsulinemia (39). In the present study, we showed that fructose improved glucose tolerance when a small amount of the sugar was included with a large amount of glucose infused intraduodenally. Thus inclusion of catalytic amounts of fructose appear to improve glucose tolerance, whereas caloric substitution of fructose for glucose is associated with insulin resistance. The explanation for this probably lies in the fact that large amounts of fructose cause large and abnormal changes in carbon flux, whereas catalytic amounts of the sugar induce enzyme changes that result in normal changes in carbon flux.

Because the human liver possesses glucokinase and the regulatory protein (35), it is possible that in humans, as well as in dogs, small amounts of fructose can increase hepatic glucose uptake by activating glucokinase. In fact, a number of abstracts (65,74–76) have recently suggested that this is the case. We reported that in normal subjects, the glycemic response to an oral glucose load was improved by the addition of a small amount of fructose (73). Likewise, under hyperinsulinemic-euglycemic conditions, infusion of a low dosage of fructose was recently reported to cause an increase in net hepatic glycogen synthesis (74). Therefore, the fructose naturally contained in meals may play a catalytic role in stimulating postprandial hepatic glucose uptake and glycogen synthesis and, as a result, serve to lower postprandial hyperglycemia and reduce the stimulus to insulin secretion. The amount of hepatic glucokinase in patients with type 2 diabetes remains at ~50% of that in normal subjects (77). It has been reported that in individuals with type 2 diabetes, the ability of hyperglycemia *per se* to suppress hepatic glucose production was nearly normalized by the addition of a catalytic amount of fructose (75), and that fructose decreases the glucose and insulin responses to an oral glucose tolerance test (76). Therefore, the addition of small amounts of fructose to glucose loads might be useful in lowering postprandial hyperglycemia in diabetic sub-

jects by increasing translocation of the available glucokinase.

ACKNOWLEDGMENTS

This research was supported by grants from the National Institutes of Health (DK-43706 and DK-20593) and the Juvenile Diabetes Foundation International.

We thank Jon Hastings and the members of the Vanderbilt Diabetes Research and Training Center Core Labs (Wanda Snead, Eric Allen, and Angelina Penalosa) for technical support. The radioimmunoassay core laboratory in the Vanderbilt Diabetes Center is supported by National Institutes of Health Grant DK-20593.

Part of this work was presented at the 57th Annual Meeting of the American Diabetes Association, Chicago, IL, 8–11 June 1997.

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