Small Amounts of Fructose Markedly Augment Net Hepatic Glucose Uptake in the Conscious Dog

Masakazu Shiota, Pietro Galassetti, Michael Monohan, Doss W. Neal, and Alan D. Cherrington

Fructose activates glucokinase by releasing the enzyme from its inhibitory protein in liver. To examine the importance of acute activation of glucokinase in regulating hepatic glucose uptake, the effect of intraportal infusion of a small amount of fructose on net hepatic glucose uptake (NHGU) was examined in 42 h–fasted conscious dogs. Isotopic ([3-^{3}H] and [U-^{14}C]glucose) and arteriovenous difference methods were used. Each study consisted of an equilibration period (–90 to –30 min), a control period (–30 to 0 min), and a hyperglycemic/hyperinsulinemic period (0–390 min). During the latter period, somatostatin (489 pmol · kg^{-1} · min^{-1}) was given, along with intraportal insulin (7.2 pmol · kg^{-1} · min^{-1}) and glucagon (0.5 ng · kg^{-1} · min^{-1}). In this way, the liver sinusoidal insulin level was fixed at four times basal (456 ± 60 pmol/l), and liver sinusoidal glucagon level was kept basal (46 ± 6 ng/l). Glucose was infused through a peripheral vein to create hyperglycemia (12.5 mmol/l plasma). Hyperglycemic hyperinsulinemia (no fructose) switched net hepatic glucose balance (micromoles per kilogram per minute) from output (11.3 ± 1.4) to uptake (14.7 ± 1.7) and net lactate balance (micromoles per kilogram per minute) from uptake (6.5 ± 2.1) to output (4.4 ± 1.5). Fructose was infused intraportally at a rate of 1.7, 3.3, or 6.7 μmol · kg^{-1} · min^{-1}, starting at 120, 210, or 300 min, respectively. In the three periods, portal blood fructose increased from <6 to 113 ± 14, 209 ± 29, and 426 ± 62 μmol/l, and net hepatic fructose uptake increased from 0.03 ± 0.01 to 1.3 ± 0.4, 2.3 ± 0.7, and 5.1 ± 0.6 μmol · kg^{-1} · min^{-1}, respectively. NHGU increased to 41 ± 3, 54 ± 5, and 69 ± 8 μmol · kg^{-1} · min^{-1}, respectively, and net hepatic lactate output increased to 11.0 ± 3.2, 15.3 ± 2.7, and 22.4 ± 2.8 μmol · kg^{-1} · min^{-1} in the three fructose periods, respectively. The amount of [^{3}H]glucose incorporated into glycogen was equivalent to 69 ± 3% of [^{3}H]glucose taken up by the liver. These data suggest that glucokinase translocation within the hepatocyte is a major determinant of hepatic glucose uptake by the dog in vivo.* ***Diabetes*** **47:867–873, 1998**

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CV, coefficient of variation; FFA, free fatty acid; NHGU, net hepatic glucose uptake; RIA, radioimmunoassay; UDP, uridine 5′-diphosphate.

Glucose uptake by the liver contributes in a major way to the disposal of alimentary glucose (1). In healthy human subjects, 20–30% of absorbed glucose is taken up by the liver, and 70% of this glucose is stored as glycogen. Liver glycogen is synthesized by both direct (glucose glucose-6-phosphate glucose-1-phosphate uridine 5′-diphosphate [UDP]-glucose glycogen) and indirect (3 carbon unit phosphoenolpyruvate glucose-6-phosphate glucose-1-phosphate UDP-glucose glycogen) pathways (2). After oral glucose ingestion in the healthy human, 50–77% of the liver glycogen is synthesized via the direct pathway. Similarly, in the conscious dog, 25–40% of a gastrointestinal glucose load is taken up by the liver; ~34% of this is stored as glycogen, and of that, 50–62% is derived from the direct pathway.

Both IDDM and NIDDM exhibit excessive postprandial hyperglycemia. In animal models of diabetes caused by insulin deficiency and/or insulin resistance, increased rates of gluconeogenesis and decreased rates of hepatic glycogen synthesis were evident (3,4). In subjects with poorly controlled IDDM or NIDDM, hepatic glycogen synthesis after a meal was 30–50% of that observed in normal subjects (5,6). When IDDM subjects were clamped at a plasma glucose concentration of 9 mmol/l and a plasma insulin concentration of 400 pmol/l, glycogen synthesis via the indirect pathway was increased by 50%, whereas glycogen synthesis through the direct pathway was decreased, relative to the rates observed in normal control subjects (7). This suggests that hepatic glycogen synthesis from glucose is defective in individuals with diabetes. Because GLUT2 expression is increased by high glucose concentrations (8), the defect in direct glycogen synthesis in diabetes probably reflects decreased glucokinase flux (from glucose to glucose-6-phosphate).

Phosphorylation of fructose is not catalyzed by glucokinase, and fructose metabolism is not regulated directly by insulin (9). 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 (10–12). 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. As reviewed by Henry and Crapo (13), however, the chronic intake of large amounts of fructose produces detrimental effects, such as insulin resistance, hyperglycemia, and hypertension. These adverse effects mean that substitution of fructose for glucose in the diet carries a significant risk (13).

On the other hand, small amounts of fructose have recently been reported to activate glucokinase in a catalytic manner. In

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the past decade, Van Shaftingen et al. (14) 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 bound, is in a conformation capable of interacting with, and inhibiting, glucokinase. Fructose-1-phosphate competes with fructose-6-phosphate for binding to the regulatory protein. The regulatory protein with fructose-1-phosphate bound is in a conformation that is not capable of interacting with glucokinase, thus glucokinase is not inhibited. Van Shaftingen et al. (14) and Agius and Peak (15) have shown that the addition of a very low concentration of fructose rapidly increases fructose-1-phosphate content in the hepatocyte and causes the release of glucokinase from its regulatory protein. Indeed, recent studies have shown that fructose at low extracellular concentrations (50–200 µmol/l) stimulated glucose phosphorylation as measured by the formation of ^3H_2O from [2-^{3}H]glucose and glycolytic flux as measured by the release of ^3H_2O from [3-^{3}H]glucose (14,16,17). These observations point to the possibility that very low amounts of fructose could have a stimulatory effect on hepatic glucose uptake in vivo and could serve as a therapeutic tool in a variety of metabolic abnormalities. At present, it is unknown whether the acute activation of glucokinase alters, in the net sense, hepatic glucose uptake and glycogen synthesis from glucose in intact animals or humans.

To evaluate the physiological impact of low amounts of fructose on hepatic glucose metabolism, we examined the effects of low-rate intraportal infusion of fructose on net hepatic glucose uptake (NHGU) and glycogen synthesis under controlled hyperglycemic and hyperinsulinemic conditions in conscious dogs.

RESEARCH DESIGN AND METHODS

Animals and surgical procedures. Experiments were performed on five 42 h–fasted mongrel dogs (17.7–27.1 kg, mean 22.4 ± 1.3 kg) of either sex that had been fed a standard meat and chow diet (31% protein, 52% carbohydrate, 11% fat, and 6% fiber based on dry weight; Kal Kan, Vernon, CA, and Wayne Dog Chow; Allied Mills, Chicago) 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 endotrachial anesthesia (15 mg/kg pentothal sodium presurgery and 1.0% 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 (1,18–21). Catheters for hormone and fructose infusion were placed in a splenic and jejunal vein. The tips of the splenic and jejunal vein catheters were placed 1 cm beyond the first site of coalescence of the catheterized vein with another vessel. 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, Chicago), their contents were aspirated, and they were flushed with saline. Angiocaths (20 gauge; Abbott) were inserted into both cephalic veins for infusion of radioactive tracers and glucose and into a saphenous vein for the infusion of somatostatin.

On the day before the experiment, the leukocyte count and hematocrit were determined. Dogs were used for an experiment only if they had *1*) a leukocyte count <18,000/mm^3, *2*) a hematocrit >38%, *3*) a good appetite, and *4*) normal stools.

Experimental design. Experiments consisted of a tracer equilibration period (–120 to –30 min), a basal sampling period (–30 to 0 min), and a hyperglycemic plus hyperinsulinemic period (0–390 min). Primed doses of NaH^{14}CO$_3$ (0.64 µCi/kg) and [U-^{14}C]glucose (1.2 µCi/kg) were given via the right cephalic vein at –120 min. Continuous infusion of [U-^{14}C]glucose (0.17 µCi/min infusion) was given via the right cephalic vein throughout the experiment. [3-^{3}H]glucose was mixed with a 50% dextrose solution and was infused via the left cephalic vein at variable rates starting at 0 min to clamp plasma glucose at 12.5 mmol/l. Somatostatin was infused (489 pmol · kg^{-1} · min^{-1}) to inhibit endogenous pancreatic insulin and glucagon secretion (0–390 min). Insulin (7.2 pmol · kg^{-1} · min^{-1}) and glucagon (0.5 ng · kg^{-1} · min^{-1}) were infused at constant rates into the portal vein starting at 0 min to raise the insulin level and to keep glucagon at a basal value. Fructose was infused into the portal vein at constant rates of 1.67 (120–210 min), 3.33 (210–300 min), and 6.67 µmol · kg^{-1} · min^{-1} (300–390 min).

Analytical procedures. Plasma glucose concentrations were determined using the glucose oxidase method in a Beckman glucose analyzer (Fullerton, CA) (22). Blood concentrations of glucose, lactate, glycerol, and alanine were determined according to the method of Lloyd et al. (23) as adapted to the Monarch 2000 centrifugal analyzer (Lexington, MA) in samples deproteinized with perchloric acid (3%). Plasma free fatty acid (FFA) concentration was determined using the Wako nonesterified fatty acid C test kit (Wako, Osaka, Japan). For the determination of plasma glucose radioactivity (^{3}H and ^{14}C), samples were deproteinized with barium hydroxide and zinc sulfate and placed over Dowex-50W (Sigma, St. Louis, MO) and Amberlite (Schweizerhall, South Plainfield, NJ) resins; the supernatant was evaporated and reconstituted in 1 ml of water, and 10 ml of liquid scintillation fluid [EcoLite (+); ICN Biomedicals, Irvine, CA] was added (18). Whole blood $^{14}CO_2$ was liberated by acidification with hydrochloric acid and trapped on chromatography paper using hyamine hydroxide. Whole blood glucose and lactate radioactivity were measured by the method described by Okajima et al. (24). Plasma arterial and hepatic vein indocyanine green concentrations were determined spectrophotometrically at 805 nm (25).

For the determination of whole blood fructose concentration, whole blood samples were deproteinized with perchloric acid and then neutralized with 10% KOH. The neutralized sample (0.5 ml) was mixed with 0.1 mol/l phosphate buffer (pH 7.4) (0.5 ml), which contained glucose oxidase (10 U/ml) and catalase (600 U/ml), and the mixture was incubated for 1 h to remove glucose. After that, fructose was measured according to the method by Beutler (26), adapted for use with the Technicon Auto Analyzer. The standard curve was obtained with solutions that contained fructose at 0, 15, 30, 60, 90, 135, 180, 250, 375, or 500 µmol/l with or without glucose (0, 7, or 14 mmol/l). The standards were handled in the same way as the blood samples. No absorbance was obtained for the standard sample, which did not contain fructose, indicating that the added glucose was completely removed by preincubation of the standard solutions with glucose oxidase and catalase. Recovery of fructose was 96.3 ± 0.5% and was linear up to 500 µmol/l. Recovery was not affected by the presence of glucose. When a given amount of fructose was mixed with whole blood, and the blood sample was treated in our normal manner, the recovery of fructose was 95.6 ± 0.8%. Therefore, the present method can be used to determine small amounts of fructose in biological samples even when they contain large amounts of glucose.

Liver samples were obtained at the end of experiments by anesthetizing dogs with sodium pentobarbital, exposing the liver by laparotomy, and freeze-clamping ~5 g of liver from each of two lobes in situ. The time elapsed from anesthesia to freeze-clamping was <2 min. The entire liver was then removed from the dog and weighed. The frozen samples were stored at –70°C until analysis. On the day of the assay, samples were powdered and homogenized, and glycogen concentrations were determined by acid hydrolysis and enzyme degradation using α-1-4,6-amyloglucosidase (27). Net incorporation of ^{3}H into glycogen was determined after liquid scintillation counting of the processed samples. Fructose-1-phosphate content in the liver was determined using the method described by Eggleston (28).

Immunoreactive plasma insulin was measured using a double-antibody procedure (interassay coefficient of variation [CV] of 11%) (29). Immunoreactive plasma glucagon was measured in plasma samples containing 500 kallikrein-inactivating units per milliliter aprotinin (Trasyrol; FBA, New York, NY) by a double-antibody radioimmunoassay (RIA) similar to that used in the insulin assay and with an interassay CV of 8% (30). Plasma cortisol was measured with the Clinical Assays Gamma-Coat RIA kit (interassay CV of 6%; Travenol-Genentech Diagnostics, Cambridge, MA). Plasma epinephrine and norepinephrine were determined by high-performance liquid chromatography, as previously described (31), with interassay CVs of 7 and 5%, respectively.

Materials. [3-^{3}H] and [U-^{14}C]glucose (New England Nuclear, Boston, MA) were used as the glucose tracers. Indocyanine green was purchased from Hynson, Westcott, and Dunning (Baltimore, MD) and was prepared in sterile water. Insulin was obtained from Squibb-Novo (Princeton, NJ), and glucagon was obtained from Eli Lilly (Indianapolis, IN). Cyclic somatostatin was purchased from Bachem (Torrance, CA). The insulin, glucagon, and somatostatin infusates were prepared with normal saline and contained 3% (vol/vol) of the dog's own plasma. Cortisol RIA kits were obtained from Micromedic Systems (Horsham, PA).

Calculations. The hepatic arterial and portal blood flows were measured with Transonic flow probes. 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, and Fa and Fp are hepatic arterial and hepatic portal vein blood or plasma flow, respectively. The percentages of hepatic conversion of glucose entering the liver to lactate and of CO_2 were obtained by dividing the [^{14}C]lactate and [^{14}C]CO_2 production rates (disintegrations per minute per kilogram per minute) by the net hepatic uptake of [^{14}C]glucose (disintegrations per minute per kilogram per minute).

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Total hepatic glycogen content was determined by multiplying the glycogen concentration (milligrams per gram of liver) by liver weight. Assuming that [^{3}H] specific activity was not diluted in the liver sinusoids, the amount of glycogen synthesized from glucose (direct pathway) during hyperglycemic hyperinsulinemia (0–390 min) was calculated by dividing the [^{3}H] radioactivity incorporated into liver glycogen by the average [^{3}H] specific activity in arterial plasma glucose during the hyperglycemic period.

Statistical analysis. Data are expressed as means ± SE. Statistical comparisons were made using repeated-measures analysis of variance. Post hoc analysis was performed using Student's paired *t* test or the unpaired *t* test (32).

RESULTS

Hepatic blood flow and hormone levels. Hepatic blood flow increased slightly by the end of the study (from 28.5 ± 2.4 ml · kg^{-1} · min^{-1} in the control period to 34.3 ± 2.7 ml · kg^{-1} · min^{-1} at the end of the study). Intraportal infusion of insulin at 7.2 pmol · kg^{-1} · min^{-1} increased the sinusoidal insulin level approximately threefold ($P < 0.05$) (Table 1). Intraportal infusion of glucagon at 0.5 ng · kg^{-1} · min^{-1} maintained the basal sinusoidal glucagon level. Arterial norepinephrine and epinephrine levels decreased by 23 and 27%, respectively, during hyperglycemic hyperinsulinemia, but the changes were not significant. Plasma cortisol levels did not change in response to treatment (Table 1).

Blood fructose levels and hepatic balance. Basal fructose levels in arterial and portal blood were 6 ± 2 and 5 ± 1 μmol/l, respectively (Fig. 1). Basal net hepatic uptake of fructose was not significant (0.02 ± 0.01 μmol · kg^{-1} · min^{-1}). The arterial and portal fructose levels and net hepatic fructose balance did not change during hyperglycemic hyperinsulinemia. Intraportal infusion of fructose at 1.7, 3.3, or 6.7 μmol · kg^{-1} · min^{-1} increased the portal fructose concentration from 4 to 113 ± 14, 209 ± 29, and 426 ± 62 μmol/l, respectively . The magnitude of the increases in arterial blood fructose were much less than those in the portal vein. Mean net hepatic fructose uptake increased from 0.03 ± 0.01 to 1.3 ± 0.4, 2.3 ± 0.7, and 5.1 ± 0.6 μmol · kg^{-1} · min^{-1} ($P < 0.05$) in the three infusion periods, respectively. The hepatic fractional extraction of the ketohexose was 52 ± 5, 48 ± 10, and 51 ± 3% in the three periods, respectively. The hepatic content of fructose-1-phosphate at the end of the experiment was 175 ± 26 nmol/g of liver.

Glucose metabolism. Hyperglycemic hyperinsulinemia switched net hepatic glucose balance from a state of output (11 ± 2 μmol · kg^{-1} · min^{-1}) to a state of uptake (14 ± 2 μmol · kg^{-1} · min^{-1}, $P < 0.05$) (Fig. 2). Intraportal infusion of fructose at 1.7 μmol · kg^{-1} · min^{-1} doubled NHGU within 10 min and eventually caused the uptake of 41 ± 3 μmol · kg^{-1} · min^{-1} ($P < 0.05$ relative to hyperglycemia alone). The further increments in fructose increased NHGU to 54 ± 5 and 69 ± 8 μmol · kg^{-1} · min^{-1} ($P < 0.05$). The fractional extractions of glucose by the liver in the four periods were 0.06 ± 0.01, 0.15 ± 0.01, 0.20 ± 0.01, and 0.23 ± 0.01, respectively. The hepatic content of glucose-6-phosphate at the end of the experiment was 330 ± 31 nmol/g of liver. Figure 3 shows the relationship between the sinusoidal blood concentration of fructose and NHGU.

Incorporation of glucose carbon into lactate and carbon dioxide. Under hyperglycemic hyperinsulinemic conditions without fructose infusion, net hepatic production of [^{14}C]lactate and $^{14}CO_2$ were equivalent to 8 ± 1 and 5 ± 3% of [^{14}C]glucose taken up by the liver (Fig. 4). In the periods of fructose infusion at 1.7, 3.3, and 6.7 μmol · kg^{-1} · min^{-1}, net hepatic production of [^{14}C]lactate was equivalent to 12 ± 2, 13 ± 3, and 17 ± 4% of net [^{14}C]glucose uptake, respectively. Hepatic $^{14}CO_2$ production increased in parallel with NHGU and was equivalent to 8 ± 3, 7 ± 2, and 7 ± 2% of net hepatic [^{14}C]glucose uptake in the three periods, respectively. The amount of [^{3}H]glucose incorporated into glycogen over the entire study was equivalent to 69 ± 3% of the [^{3}H]glucose taken up by the liver (Table 2).

Blood lactate and net hepatic lactate balance. As shown in Fig. 5, the hyperglycemic hyperinsulinemic clamp shifted net hepatic lactate balance from an uptake of 6.5 ± 2.1 to production of 4.4 ± 1.5 μmol · kg^{-1} · min^{-1} and resulted in a rise in blood lactate from 0.7 ± 0.2 to 1.1 ± 0.6 mmol/l. Intraportal infusion of fructose at 1.7, 3.3, 6.7 μmol · kg^{-1} · min^{-1} further increased net hepatic lactate production to 11.1 ± 3.4, 14.8 ± 2.8, and 22.3 ± 4.9 μmol · kg^{-1} · min^{-1}, respectively. Net lactate output was linearly and positively correlated with net glucose uptake.

TABLE 1
Arterial and portal plasma levels of insulin and glucagon and the arterial levels of catecholamines and cortisol before and during intraportal fructose (IPF) infusion in 42 h–fasted conscious dogs under conditions of hyperglycemia plus hyperinsulinemia (i.e., in the presence of peripheral glucose infusion to double the hepatic glucose load and a threefold increase in insulin)

	Control period	0 μmol · kg^{-1} · min^{-1} IPF		1.67 μmol · kg^{-1} · min^{-1} IPF		3.33 μmol · kg^{-1} · min^{-1} IPF		6.67 μmol · kg^{-1} · min^{-1} IPF	
		90 min	120 min	150 min	210 min	240 min	300 min	330 min	390 min
Insulin (pmol/l)									
Arterial	66 ± 12	162 ± 30*	174 ± 30*	180 ± 36*	192 ± 24*	198 ± 24*	192 ± 24*	222 ± 36*	234 ± 24*
Portal	204 ± 18	690 ± 96*	714 ± 96*	582 ± 60*	624 ± 90*	762 ± 60*	684 ± 102*	732 ± 72*	546 ± 72*
Sinusoidal	162 ± 18	432 ± 54*	468 ± 54*	432 ± 48*	462 ± 72*	486 ± 78*	498 ± 72*	486 ± 66*	438 ± 60*
Glucagon (ng/l)									
Arterial	41 ± 4	42 ± 5	40 ± 5	38 ± 6	37 ± 4	34 ± 5	33 ± 6	34 ± 6	33 ± 5
Portal	9 ± 7	61 ± 4	68 ± 4	62 ± 11	56 ± 8	64 ± 7	56 ± 10	57 ± 9	55 ± 5
Sinusoidal	53 ± 6	52 ± 5	57 ± 5	53 ± 8	48 ± 6	52 ± 6	47 ± 8	50 ± 5	46 ± 5
Norepinephrine (pmol/l)	1135 ± 136	910 ± 106	845 ± 106	987 ± 118	833 ± 24	863 ± 24	822 ± 47	981 ± 47	851 ± 100
Epinephrine (pmol/l)	431 ± 55	300 ± 60	327 ± 60	404 ± 120	191 ± 55*	256 ± 49*	284 ± 82*	267 ± 60*	224 ± 49*
Cortisol (nmol/l)	5.0 ± 0.8	5.5 ± 1.4	3.9 ± 1.4	3.3 ± 0.6	5.8 ± 1.4	3.9 ± 0.8	5.2 ± 0.3	4.1 ± 0.6	4.7 ± 1.1

Data are means ± SE for five dogs, except data for control period, which are means of values at –30 and 0 min. *Significantly different from the control period ($P < 0.05$).

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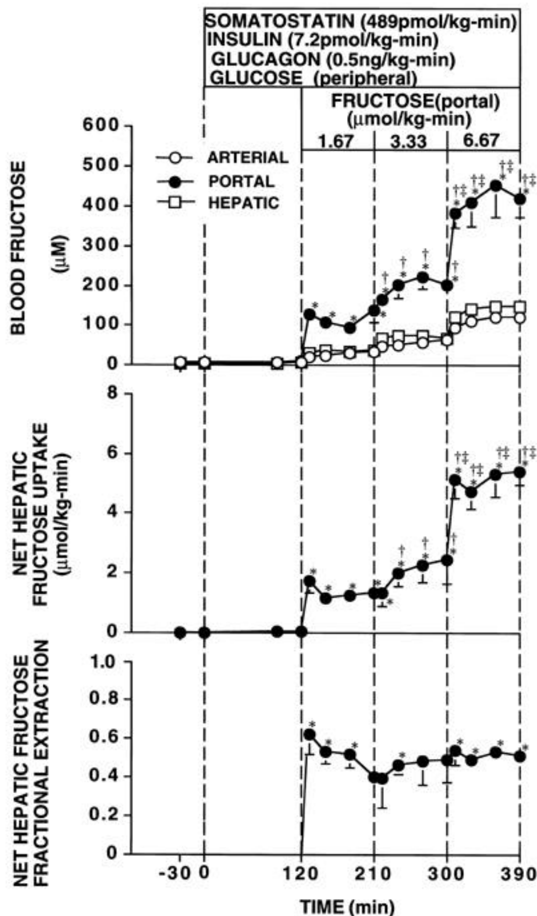

FIG. 1. Arterial and portal blood fructose levels and changes in net hepatic fructose balance before and during intraportal fructose infusion in 42 h–fasted conscious dogs in the presence of peripheral glucose infusion to double the hepatic glucose load and a threefold increase in insulin. These results by statistical analysis were the same in the fructose levels in the artery, portal vein, and hepatic vein. Values are means ± SE for five dogs. *Significantly different from the control period ($P < 0.05$); †significantly different from the value at 210 min ($P < 0.05$); ‡significantly different from the value at 300 min ($P < 0.05$).

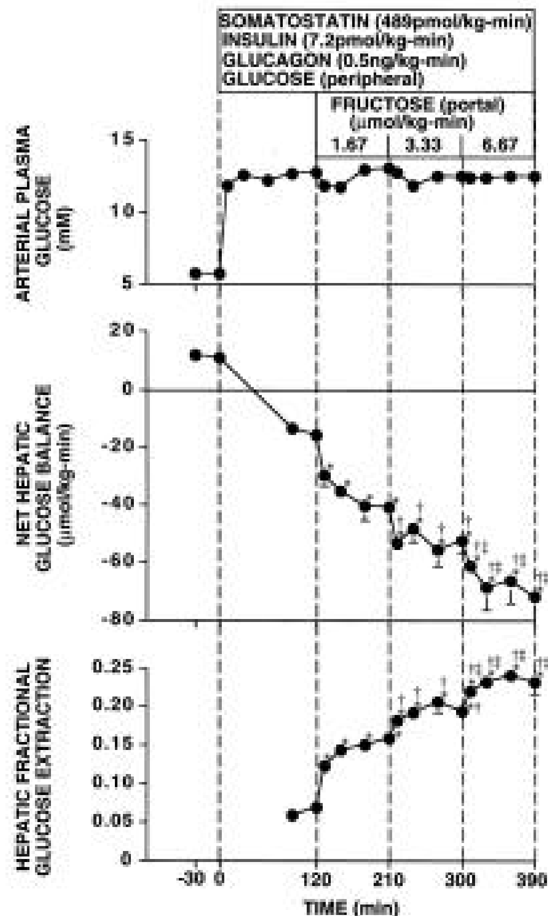

FIG. 2. Arterial plasma glucose levels, net hepatic glucose balances, and hepatic fractional glucose extractions before and during intraportal fructose infusion in 42 h–fasted conscious dogs in the presence of peripheral glucose infusion to double the hepatic glucose load and a threefold increase in insulin. Values are means ± SE for five dogs. *Significantly different from the control period; †significantly different from the value at 210 min; ‡significantly different from the value at 300 min.

Blood alanine and net hepatic alanine uptake. The arterial alanine level and net hepatic alanine uptake were unchanged from basal (375 ± 54 µmol/l and 3.0 ± 0.4 µmol · $kg^{-1} \cdot min^{-1}$) with hyperglycemic hyperinsulinemia (323 ± 37 µmol/l and 3.0 ± 0.2 µmol · $kg^{-1} \cdot min^{-1}$). During fructose infusion at 1.7, 3.3, and 6.7 µmol · $kg^{-1} \cdot min^{-1}$, net hepatic alanine uptake decreased from 2.9 ± 0.2 to 2.6 ± 0.2, 2.3 ± 0.3, and 1.8 ± 0.7 µmol · $kg^{-1} \cdot min^{-1}$, respectively (data not shown). The arterial alanine level did not change (382 ± 53 µmol/l at the end of study) significantly.

Blood glycerol and net hepatic glycerol uptake. The hyperglycemic hyperinsulinemic clamp decreased arterial glycerol and net hepatic glycerol uptake from 65 ± 6 µmol/l and 1.3 µmol · $kg^{-1} \cdot min^{-1}$ to 27 ± 3 µmol/l and 0.4 ± 0.1 µmol · $kg^{-1} \cdot min^{-1}$, respectively. Intraportal fructose infusion did not affect these parameters (34 ± 6 µmol/l and 0.7 ± 0.2 µmol · $kg^{-1} \cdot min^{-1}$ at the end of study) (data not shown).

Blood FFA and net hepatic FFA uptake. Hyperglycemic hyperinsulinemia decreased plasma FFA level and net hepatic FFA uptake from 740 ± 9 and 2.5 ± 0.3 to 15 ± 2 mmol/l and 0.45 ± 0.16 µmol · $kg^{-1} \cdot min^{-1}$, respectively. Fructose infusion produced no further effects (8 ± 2 µmol/l and 0.32 ± 0.11 µmol · $kg^{-1} \cdot min^{-1}$ at the end of study) (data not shown).

DISCUSSION

The results of the present study demonstrate that a small amount of fructose can augment NHGU more than threefold in the presence of hyperinsulinemia and hyperglycemia resulting from peripheral glucose infusion. The glucose that entered the liver was stored in glycogen (69%) and released as lactate (17%) or oxidized (8%). Almost all (90%) of the stored glycogen was synthesized via the direct pathway.

The liver, kidney, and intestine possess the enzymes, fructokinase, aldolase B, and triokinase that catalyze fructose metabolism (9). When fructose was infused at 1.7, 3.3, and 6.7 µmol · $kg^{-1} \cdot min^{-1}$, the appearance of fructose in the portal blood accounted for 91, 81, and 93% of the infused hexose. In experiments in which fructose was infused through a peripheral vein in 42 h–fasted dogs, net gut fructose uptake accounted for ~10% of the fructose reaching the gut. It is likely, therefore, that our failure to observe complete recovery of the infused fructose is explained by the metabolism of a small amount of the fructose in the blood by the gut.

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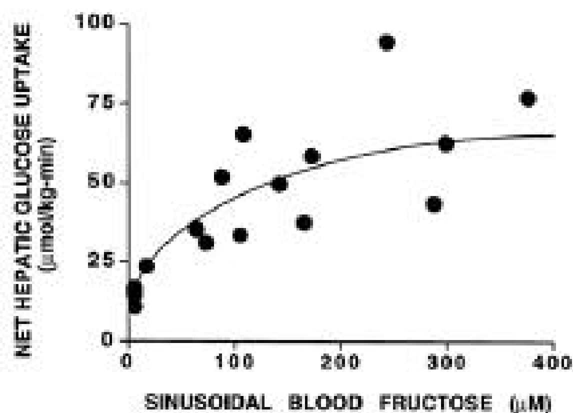

FIG. 3. Relationship between NHGU and the sinusoidal blood fructose concentration.

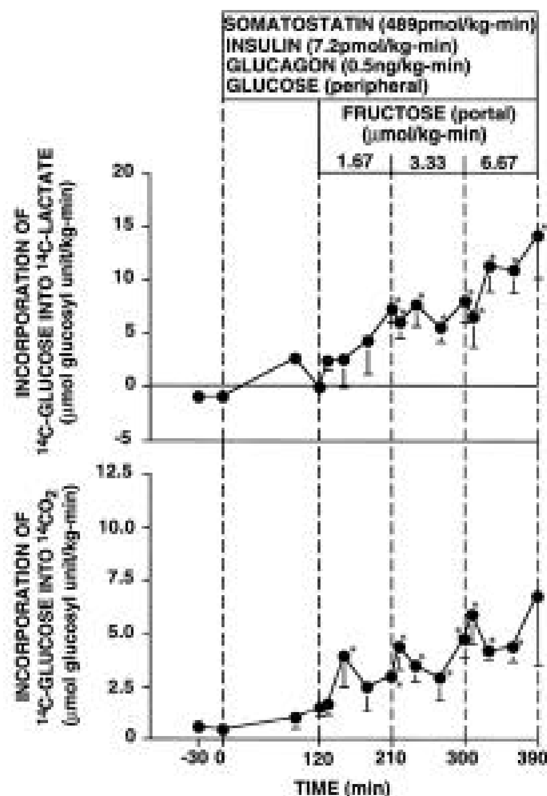

FIG. 4. Net hepatic [^{14}C]lactate and $^{14}CO_2$ production before and during intraportal fructose infusion in 42 h–fasted conscious dogs in the presence of peripheral glucose infusion to double the hepatic glucose load and a threefold increase in insulin. Values are means ± SE for five dogs. *Significantly different from the control period (P < 0.05).

In humans, approximately one-half of the fructose given by prolonged intravenous infusion is taken up by the splanchnic tissues, with the liver accounting for >75% of this removal (9,33). In the present study, the hepatic fractional extraction of fructose (first pass extraction) was 50% over a wide range of sinusoidal fructose concentrations (60–300 µmol/l) (Fig. 1). On the other hand, overall net hepatic fructose uptake could account for 77, 70, and 77% of the fructose infused, indicating that almost three-fourths of the infused hexose was eventually cleared by the liver. These results confirm that the liver is the major site of fructose clearance from blood and indicate that this is so even when blood fructose levels are <500 µmol/l.

Since the presence of GLUT2 in the liver allows a rapid equilibration of the intracellular glucose level with the extracellular glucose level (34,35), net hepatic glucose flux represents a balance between glucokinase flux and glucose-6-phosphatase flux. Wals and Katz (17) showed that low concentrations of fructose (50–200 µmol/l) increased both glucose phosphorylation and recycling between glucose and glucose-6-phosphatase proportionally in isolated rat hepatocytes, indicating that fructose did not inhibit glucose-6-phosphatase flux. Niewoehner et al. (36) reported that the administration of a wide range of fructose loads (0.28–2.8 mmol/kg) to intact animals did not affect glucose-6-phosphatase activity, despite a marked increase in the intracellular content of fructose-1-phosphate in the liver. In hepatocyte studies, on the other hand, glucokinase was activated by only 50–200 nmol/l of intracellular fructose-1-phosphate, which was associated with 100–200 µmol/l of extracellular fructose (14,15). In the present study, the hepatic sinusoidal concentration of fructose during the intraportal infusion of the ketohexose was in the range of 60–300 µmol/l (Fig. 2). Despite such a low concentration of fructose and a low fructose-to-glucose ratio in plasma (0.005–0.02), fructose was taken up very efficiently by the liver, and the intracellular fructose-1-phosphate content at the end of the experiment was 175 ± 26 nmol/g of liver, which was significantly higher than the basal content of fructose-1-phosphate (102 ± 13 nmol/g liver). In addition, we have recently shown that the dog liver, like the rat liver, possesses the regulatory protein of glucokinase and that it is located in the nucleus (M.S., P.G., T.L. Jetton, M.A. Magnuson, A.D.C., unpublished observations). Further, we have shown that glucokinase is colocalized with the regulatory protein in the nucleus under basal conditions and that its translocation to the cytosol increases in response to fructose infusion (M.S., P.G., T.L. Jetton, M.A. Magnuson, A.D.C., unpublished observations). It is likely, therefore, that fructose can augment NHGU in the conscious dog by causing translocation, and thus activation, of glucokinase.

In our previous study (20,21), in which 42 h–fasted dogs were exposed to hyperinsulinemia (fourfold basal) and hyperglycemia (13 mmol/l in arterial plasma) for 4 h, NHGU during steady state was 13–16 µmol · kg^{-1} · min^{-1}, and the glycogen synthetic rate was 13–15 µmol glucose equivalent · kg^{-1} · min^{-1}. The direct pathway was responsible for 54–58% of the glycogen synthesis. In the presence of fructose (500 µmol/l in hepatic portal blood), NHGU reached 66 µmol · kg^{-1} · min^{-1}. Hepatic glycogen content at the end of the experiments was 496 µmol glucose equivalent/g liver (Table 2). Previously, we showed that the hepatic glycogen content of the 42 h–fasted dog was ~139 ± 6 µmol glucose equivalent/g (19). Therefore, the increment in hepatic glycogen content was 356 µmol glucose equivalent/g liver during 390 min of experimental period. The average glycogen synthetic rate was thus 31 µmol glucose equivalent · kg^{-1} · min^{-1}. The incorporation of [3-^{3}H]glucose into hepatic glycogen, which allows estimation of the glycogen synthesis from glucose via the direct pathway, indicated that 324 µmol glucose equivalent/g (Table 2) were stored in glycogen via the direct pathway. Thus, at least 90% of the accumulated glycogen was synthesized via

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TABLE 2
Glycogen content and incorporated glucose into glycogen at the end of the experiment

Parameters	Values
Liver weight (g/kg)	34.0 ± 1.6
Glycogen content (μmol glucosyl unit/g liver)	496 ± 29
[^{3}H]glucose uptake (μmol glucosyl unit/g liver)	467 ± 36
[^{3}H]glucose incorporation into glycogen (μmol glucosyl unit/g liver)	324 ± 16
% of glucose taken up deposited in glycogen (%)	69.4 ± 3.3

Data are means ± SE for five dogs.

the direct pathway. Therefore, activation of glucokinase increased net glycogen synthesis and the relative contribution of the direct pathway to glycogen accumulation.

Net glycogen deposition depends on the activities of glycogen synthase and phosphorylase. The effects of fructose administration on phosphorylase activity are controversial (33,37–39). Gergely et al. (37) and Bollen et al. (38) observed an activation of phosphorylase and an inhibition of phosphorylase phosphatase by fructose-1-phosphate in liver extracts. In contrast, Kaufmann and Froesch (39) showed an inhibition of phosphorylase by fructose-1-phosphate. On the other hand, an activation of hepatic glycogen synthase has been repeatedly observed with fructose administration in in vivo and in vitro studies (33). Several studies (40,41) have suggested that activation of glycogen synthase by the administration of relatively small fructose loads to intact animals was secondary to increased glucose-6-phosphate, a potent activator for glycogen synthase (42). The glucose-6-phosphate content at the end of the experiment in the present study was 330 ± 31 μmol/g of liver (n = 5), which is higher than the level of this metabolite content (250 ± 45 μmol/g of liver; n= 5) in the liver of dogs exposed to hyperglycemia (12 mmol/l) for 300 min in the presence of similar insulin and glucagon levels but in the absence of fructose. It is possible, therefore, that fructose can activate glycogen synthase via an increase in intracellular content of glucose-6-phosphate by increasing glucose phosphorylation.

The intraportal infusion of a small amount of fructose increased net hepatic lactate production (Fig. 5), indicating that fructose stimulated glycolysis in the liver. Pyruvate kinase and phosphofructokinase are the regulatory steps of glycolytic flux. Low fructose loads to intact animals (43) or isolated hepatocytes (16) increased intracellular content of fructose-2,6-diphosphate, a potent activator of phosphofructokinase (44). The effect might be secondary to an increase in glucose-6-phosphate content because fructose-induced activation of glycolysis was not observed in rat hepatocytes incubated in the presence of mannoheptulase where glucokinase was inhibited (16). Pyruvate kinase in the perfused liver has been reported to be activated by fructose-1-phosphate (0.5–10 mmol/l) or by a fructose load (1–5 mmol/l) (45). Therefore, the increased glycolytic flux observed during intraportal infusion of fructose in the present study may also be secondary to an increase in the intracellular glucose-6-phosphate content secondary to the activation of glucokinase.

The metabolic characteristics of fructose have been compared with those of glucose (9,33). While fructose is a better substrate for gluconeogenesis than lactate, the ketohexose is

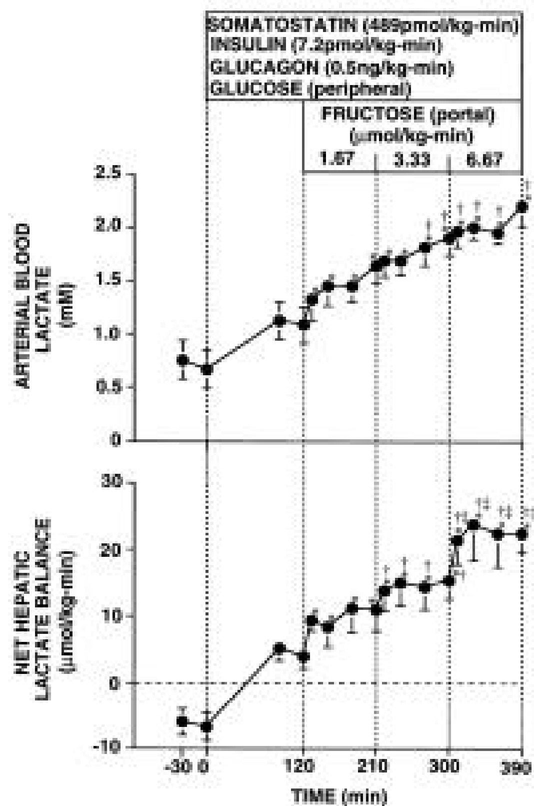

FIG. 5. Arterial blood lactate levels and net hepatic lactate balances before and during intraportal fructose infusion in 42 h–fasted conscious dogs in the presence of peripheral glucose infusion to double the hepatic glucose load and a threefold increase in insulin. *Significantly different from the control period (P < 0.05); †significantly different from the value at 210 min (P < 0.05); ‡significantly different from the value at 300 min (P < 0.05).

a better precursor of hepatic glycogen than glucose, and fructolysis is several times faster than glycolysis. As a result, the extent of the increase in blood glucose after fructose feeding is much smaller than that after feeding of the same amount of glucose. For this reason, the use of fructose as a substitute for dietary sucrose and dextrose has been examined as a potential therapeutic intervention in individuals with diabetes. As a result, however, the effects of fructose on glucose metabolism have been determined in studies that were performed after several days or weeks of the feeding of these carbohydrates (9,13,46). Such studies showed that long-term consumption of relatively large amounts of fructose or sucrose reduced the conversion of glucose to glycogen, glucose oxidation, and fatty acid synthesis from glucose in the liver. The use of glucose for glycogen and/or lipid synthesis in muscle and adipose tissue also decreased after long-term sucrose or fructose feeding. In contrast, studies using isolated hepatocytes or perfused liver (14,16,17,47) in which the acute effects of low levels of fructose were examined showed that fructose stimulated glucose phosphorylation, glycolysis, and the incorporation of glucose into glycogen within the liver. In the present study, we showed in conscious dogs that small amounts of fructose can acutely and markedly augment NHGU to a rate (69 μmol · kg^{-1} · min^{-1}) more than twice that seen in the normal postprandial state (1). Since the human

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liver possesses glucokinase and the regulatory protein (14), it is possible that in humans, as well as in dogs, small amounts of fructose can increase hepatic glucose uptake by activating glucokinase. 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. If small amounts of fructose can augment hepatic glucose uptake, the inclusion of small amounts of fructose in meals may be useful in lowering postprandial hyperglycemia in subjects with diabetes.

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