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Inclusion of low amounts of fructose with an intraportal glucose load increases net hepatic glucose uptake in the presence of relative insulin deficiency in dog

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Shiota, Masakazu, Pietro Galassetti, Kayano Igawa, Doss W. Neal, and Alan D. Cherrington. Inclusion of low amounts of fructose with an intraportal glucose load increases net hepatic glucose uptake in the presence of relative insulin deficiency in dog. *Am J Physiol Endocrinol Metab* 288: E1160–E1167, 2005. First published January 25, 2005; doi:10.1152/ajpendo.00391.2004.—The effect of small amounts of fructose on net hepatic glucose uptake (NHGU) during hyperglycemia was examined in the presence of insulinopenia in conscious 42-h fasted dogs. During the study, somatostatin (0.8 $\mu g \cdot kg^{-1} \cdot min^{-1}$) was given along with basal insulin (1.8 $pmol \cdot kg^{-1} \cdot min^{-1}$) and glucagon (0.5 $ng \cdot kg^{-1} \cdot min^{-1}$). After a control period, glucose (36.1 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) was continuously given intraportally for 4 h with (2.2 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) or without fructose. In the fructose group, the sinusoidal blood fructose level (nmol/ml) rose from <16 to 176 ± 11. The infusion of glucose alone (the control group) elevated arterial blood glucose (μmol/ml) from 4.3 ± 0.3 to 11.2 ± 0.6 during the first 2 h after which it remained at 11.6 ± 0.8. In the presence of fructose, glucose infusion elevated arterial blood glucose (μmol/ml) from 4.3 ± 0.2 to 7.4 ± 0.6 during the first 1 h after which it decreased to 6.1 ± 0.4 by 180 min. With glucose infusion, net hepatic glucose balance ($\mu mol \cdot kg^{-1} \cdot min^{-1}$) switched from output (8.9 ± 1.7 and 13.3 ± 2.8) to uptake (12.2 ± 4.4 and 29.4 ± 6.7) in the control and fructose groups, respectively. Average NHGU ($\mu mol \cdot kg^{-1} \cdot min^{-1}$) and fractional glucose extraction (%) during last 3 h of the test period were higher in the fructose group (30.6 ± 3.3 and 14.5 ± 1.4) than in the control group (15.0 ± 4.4 and 5.9 ± 1.8). Glucose 6-phosphate and glycogen content (μmol glucose/g) in the liver and glucose incorporation into hepatic glycogen (μmol glucose/g) were higher in the fructose (218 ± 2, 283 ± 25, and 109 ± 26, respectively) than in the control group (80 ± 8, 220 ± 31, and 41 ± 5, respectively). In conclusion, small amounts of fructose can markedly reduce hyperglycemia during intraportal glucose infusion by increasing NHGU even when insulin secretion is compromised.

diabetes mellitus; hyperglycemia; hyperinsulinemia

INDIVIDUALS WITH TYPE 2 DIABETES MELLITUS exhibit excessive postprandial hyperglycemia with a defect in meal- or glucose-induced suppression of endogenous glucose production (18, 21, 28, 34). Several studies (21, 28, 34) have demonstrated that the greater net splanchnic glucose release in diabetic compared with nondiabetic subjects after glucose injection was due to excessive endogenous glucose production rather than lower initial splanchnic extraction of the 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. (15) and Ludvik et al. (30) compared the splanchnic glucose uptake during a euglycemic hyperinsulinemic clamp in diabetic and nondiabetic subjects. Ludvik et al., but not DeFronzo et al. (15), found decreased splanchnic glucose uptake in diabetic subjects. It is known, however, that in the presence of euglycemia and hyperinsulinemia, there is minimal splanchnic glucose uptake (14, 15, 23, 42). Thus the size of the signal in the above studies was very small. Hyperglycemia combined with hyperinsulinemia, on the other hand, substantially increases glucose uptake by the liver (14, 23, 42). Recently, Basu et al. (5) carried out a hyperglycemic and hyperinsulinemic clamp study in the human and showed that the increase in splanchnic glucose uptake, as well as the suppression of splanchnic glucose production, was reduced in individuals with type 2 diabetes compared with 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 (5). A reduced rate of hepatic glycogen synthesis from glucose via the direct pathway has been reported in other studies (31). The same alterations in hepatic glucose metabolism have been found in various animal models of diabetes (3, 22, 23). GLUT 2 expression is increased by high glucose concentrations (8). Because the presence of GLUT2 in the liver allows a rapid equilibration of the intracellular glucose level with the extracellular glucose level (39), net hepatic glucose flux represents a balance between glucokinase and glucose 6-phosphatase flux. Rossetti et al. (44) demonstrated that an inhibition of net hepatic glucose production occurs when the blood glucose levels are raised as a result of increased glucokinase flux. Therefore, it is likely that the excessive postprandial hyperglycemia evident in diabetic subjects is, in part, due to 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 catalytic manner. Van Shaftingen et al. (49) 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

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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. We showed that intraportal infusion of small amount of fructose at 1.7, 3.3, or 6.7 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, which raised the portal blood fructose concentration from <6 (basal) to 113, 209, and 426 μmol/l, respectively, increased NHGU from 15 to 41, 54, and 69 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, respectively, during a hyperglycemic/hyperinsulimenic clamp in 42-h fasted dogs (45). 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. The inclusion of small amounts of fructose with the intraduodenal glucose load augmented NHGU, increased hepatic glycogen synthesis via the direct pathway, and augmented hepatic glycolysis. As a result, postprandial hyperglycemia and insulin release were reduced (46). It has also been shown that fructose administration stimulates hepatic glycogen synthesis in the presence of hyperinsulinemia in healthy subjects (43). These studies demonstrated that small amounts of fructose markedly stimulate hepatic glucose uptake with a resulting increase in its storage as glycogen and of its catabolism by glycolysis. Because insulin stimulates glycogen synthesis by activating glycogen synthase (6, 19) and glycolysis by increasing fructose 2,6-phosphate levels (4, 25), it is possible that the effect of insulin to stimulate glucose 6-phosphate disposal exerts a permissive effect on the stimulation of glucokinase flux by fructose. It has been reported, on the other hand, 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 (24) and that fructose decreases the glucose and insulin responses to an oral glucose tolerance test (36). It is likely that small amounts of fructose could stimulate not only hepatic glucose phosphorylation but also glycogen synthesis and glycolysis in the liver independently from the action of insulin.

To evaluate whether a catalytic amount of fructose can lessen postprandial hyperglycemia, even in the absence of an increase in plasma insulin, we examined the effects of the inclusion of small amounts of fructose with an intraportal glucose load on the resulting increments in plasma glucose under euinsulinemic conditions in conscious dogs.

RESEARCH DESIGN AND METHODS

Animals and surgical procedures. Experiments were performed on 10 42-h fasted mongrel dogs (19.2∼26.6 kg, mean 23.1 ± 0.6 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, and 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 Accrediation 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 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 (45, 46). 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 *1*) a leukocyte count <18,000/mm³, *2*) a hematocrit >38%, *3*) a good appetite, and *4*) 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, somatostatin was infused (489 $pmol \cdot kg^{-1} \cdot min^{-1}$) to inhibit endogenous pancreatic insulin and glucagons secretion. Insulin (1.8 $pmol \cdot kg^{-1} \cdot min^{-1}$) and glucagon (0.5 $ng \cdot kg^{-1} \cdot min^{-1}$) were infused at constant rates into the portal vein to keep the hormones at basal values. Glucose was infused into the portal vein constantly at 36.1 $\mu mol\ glucose \cdot kg^{-1} \cdot min^{-1}$ with ($n = 5$) or without ($n = 5$) fructose infusion into the portal vein at 2.22 $\mu mol \cdot kg^{-1} \cdot min^{-1}$.

Analytic procedures. Plasma glucose concentrations were determined using the glucose oxidase method in a Beckman glucose analyzer (Beckman Instruments, Fullerton, CA) (26). Blood concentrations of lactate, glycerol, alanine, and 3-hydroxybutyric acid were determined according to the method of Lloyd et al. (29) adapted to the Monarch 2000 centrifugal analyzer (Lexington, MA) in samples deproteinized with perchloric acid. Blood fructose concentrations were determined as previously reported (45). Plasma free fatty acid (FFA) concentration was determined using the Wako nonesterified fatty acid C test (Wako, Osaka, Japan). For determination of plasma glucose ^3H radioactivity, samples were deproteinized with barium hydroxide and zinc sulfate, and the supernatant was evaporated and reconstituted in 1 ml of water, and 10 ml of liquid scintillation fluid [EcoLite (+); ICN Biomedicals, Irvine, CA] were added (45, 46).

Liver samples were obtained at the end of experiments by anesthetizing the dog with pentobarbital sodium, exposing the liver by laparotomy, and freeze-clamping liver in situ in less than 2 min. The entire liver was then removed from the dog and weighed. The frozen samples were stored at −70°C until subsequent analysis. On the day of the assay, samples were powdered, and the glycogen was extracted and purified as previously described (45, 46). The glycogen concentrations were determined by acid hydrolysis and enzyme degradation using α1-4,6-amyloglucosidase (11). Glucose 6-phosphate contents were determined according to the method of Michal (33).

Immunoreactive plasma insulin glucagons and cortisol were determined as previously shown (45, 46).

Calculations. The hepatic arterial and portal blood flow were measured by Transonic flow probes. Net hepatic substrate balance was calculated using the formula [H(Fa + Fp) − AFa − PFp], where H, A, and P are the arterial, portal vein, and hepatic vein substrate concentrations, 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. Total hepatic glycogen content was determined by multiplying the glycogen concentration (μmol/g liver) by liver weight. The liver sinusoidal plasma [^3H]glucose or unlabled glucose concentrations were calculated using the formula [(AgFa + PgFp)/(Fa + Fp) and (^{3}Hfa/Ag + ^{3}Hfp/Pg)/(Fa + Fp)]. The amount of glycogen synthesized from glucose by the direct pathway (glucose → glucose 6-phosphate → glucose 1-phosphate → UDP-glucose → glycogen) during the test period was calculated by dividing the ^3H radioactivity incorporation into liver glycogen by the average ^3H specific activity in the sinusoidal plasma glucose during the test period.

Statistical analysis. Data are expressed as means ± SE. A one-way ANOVA for repeated measures was used to analyze changes over time. 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.

Table 1. *Sinusoidal plasma levels of insulin and glucagon and arterial plasma levels of cortisol before and during an intraportal infusion of glucose with and without continuous intraduodenal infusion of fructose in 42-h-fasted conscious dogs*

Group	Control period	Infusion period 30	60	120	180	240
Insulin, pmol/l						
Control	115±12	107±23	100±19	137±36	163±36	160±42
Fructose	99±22	118±23	104±17	129±35	110±16	104±13
Glucagon, ng/l						
Control	50±2	46±3	42±2	45±3	49±5	45±3
Fructose	56±7	53±4	52±4	55±4	54±3	53±4
Cortisol, nmol/l						
Control	41±8	72±25	63±22	52±14	55±17	44±8
Fructose	50±14	44±14	61±25	52±14	41±11	39±19

Time, min. Data are means ± SE; n = 5 dogs for each group.

RESULTS

Hormonal concentrations. Plasma levels of insulin and glucagon were similar in the control and the fructose groups and were maintained at the basal values during the test period in both groups (Table 1). Plasma cortisol levels were basal and similar in both groups (Table 1).

Blood fructose concentration, hepatic fructose balance, and hepatic fructose fractional extraction. Basal fructose levels in arterial and portal blood were similar in both the control (14 ± 3 and 20 ± 3 μmol/l, respectively) and the fructose groups (12 ± 4 and 16 ± 4 μmol/l, respectively; Fig. 1). The intraportal infusion of glucose alone did not alter the fructose concentrations in arterial (17 ± 4 μmol/l) or hepatic portal venous blood (26 ± 3 μmol/l). The intraduodenal fructose infusion at 2.22 μmol·kg^{-1}·min^{-1} increased arterial and portal concentrations of the sugar to 56 ± 6 and 154 ± 39 μmol/l, respectively, by 10 min, after which they averaged 79 ± 6 and 205 ± 19 μmol/l, respectively. The average rate of net hepatic fructose uptake was 2.2 μmol·kg^{-1}·min^{-1}, which was equivalent to the infusion rate into the portal vein. The average net hepatic fractional extraction of fructose during the infusion period was 50%.

Plasma glucose concentrations, hepatic glucose balance, and fractional extraction. In response to intraportal glucose infusion alone, the arterial and portal blood glucose levels (mmol/l) rose from 4.3 ± 0.3 and 4.2 ± 0.3 to 11.2 ± 0.6 and 12.5 ± 0.5, respectively, by 120 min and thereafter averaged 11.6 ± 0.8 and 13.4 ± 0.7, respectively (Fig. 2). In the presence of intraportal fructose infusion, arterial plasma glucose levels (mmol/l) rose from 4.3 ± 0.2 and 4.2 ± 0.1 to 7.4 ± 0.6 and 8.5 ± 0.5, respectively, by 60 min, after which they gradually fell to 6.1 ± 0.4 and 7.7 ± 0.5, respectively, by 240 min. The increment in the arterial plasma glucose level at 240 min in the presence of fructose infusion was only 30% of that seen in the absence of fructose infusion.

Before the start of the infusion period, net hepatic glucose outputs (μmol·kg^{-1}·min^{-1}) were similar in the presence (8.9 ± 1.7) and absence (13.3 ± 2.8) of fructose infusion (Fig. 2). In the control group, net hepatic glucose production was completely shut down by 20 min (−0.7 ± 2.0 μmol·kg^{-1}·min^{-1}), after which hepatic glucose uptake rates increased gradually, eventually reaching 18.2 ± 3.4 μmol·kg^{-1}·min^{-1}. In the presence of fructose infusion, the increase in NHGU was significantly greater, reaching 30.3 ± 3.6 μmol·kg^{-1}·min^{-1} by 240 min, despite substantially lower arterial and portal glucose levels. Net hepatic fractional extraction of glucose at 240 min reached 7.4% in the absence of fructose infusion but was twice that (14.5%) in its presence.

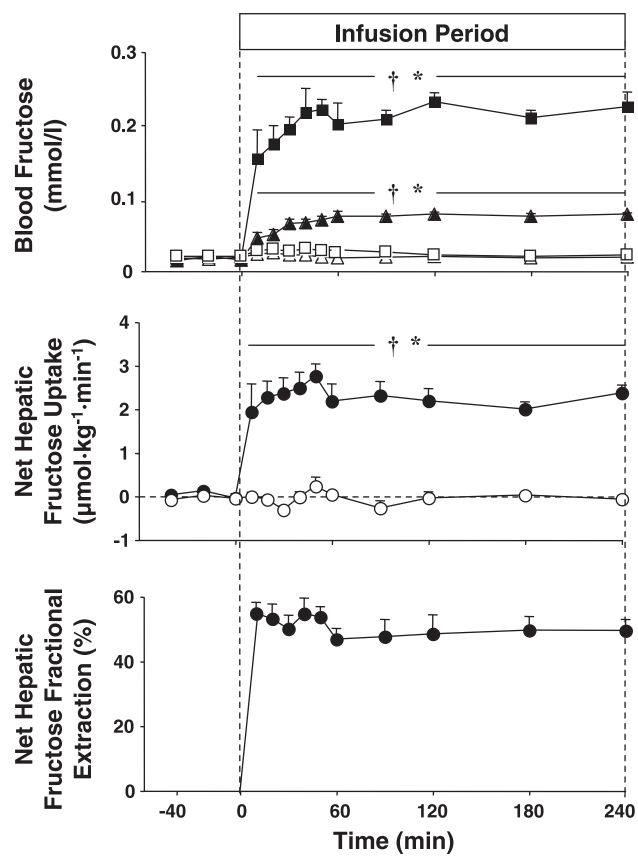

Fig. 1. Arterial (triangles) and portal vein (squares) blood fructose levels and changes in net hepatic fructose balance before and during continuous intraportal infusion of glucose at 44.4 μmol·kg^{-1}·min^{-1} with (filled symbols) and without (open symbols) continuous intraportal infusion of fructose at 2.22 μmol·kg^{-1}·min^{-1} in 42-h fasted conscious dogs. Data represent means ± SE, n = 5 for each group. †Significantly different from control period in identical vessel ($P < 0.05$). *Significantly different from the corresponding value in control group ($P < 0.05$).

Lactate concentration and metabolism. During the control period, the arterial blood lactate concentrations were similar in the two groups, although the net hepatic lactate uptake rates were somewhat higher in the fructose group (Fig. 3). In response to intraportal glucose infusion, the liver switched from net uptake to net output of lactate in both groups. The increment in blood lactate and in net hepatic lactate output was higher in the fructose group than in the control group.

Alanine, glycerol, and NEFA concentrations and metabolisms. The arterial blood alanine concentration rose slightly during the intraportal infusion of glucose alone and somewhat more during combined intraportal glucose and fructose infusion (Table 2). Net hepatic alanine uptake ($\mu mol \cdot kg^{-1} \cdot min^{-1}$) rose slightly in the presence of fructose but not in the absence of fructose. The hepatic fractional extraction of alanine decreased significantly in both groups. In response to an intraportal glucose given in the presence and absence of fructose infusion, the arterial blood glycerol and plasma NEFA concentrations and net hepatic glycerol and NEFA uptake did not change (Table 2).

Hepatic glucose 6-phosphate content and hepatic disposition of glucose. At the end of the experiment, hepatic glucose 6-phosphate content (218 ± 104 nmol/g) in the fructose group was 2.5 times higher than it was in the control group (80 ± 8 nmol/g; Fig. 4). Previously, we showed that the hepatic glycogen content of the 42-h fasted dog was 139 ± 6 μmol glucose equivalents/g tissue (35). At the end of the experiment in the control group, the hepatic glycogen content was 220 ± 31 μmol glucose equivalents/g (Fig. 5). Glycogen synthesis via the direct pathway accounted for 41 ± 5 μmol glucose equivalents/g. In the fructose group, on the other hand, the hepatic glycogen content at the end of experiment was 283 ± 25 μmol glucose equivalents/g liver and glycogen synthesis via the direct pathway contributed 109 ± 26 μmol glucose equivalents/g. Therefore, the increment in glycogen content caused by fructose is due to the increase in glycogen synthesis via the direct pathway.

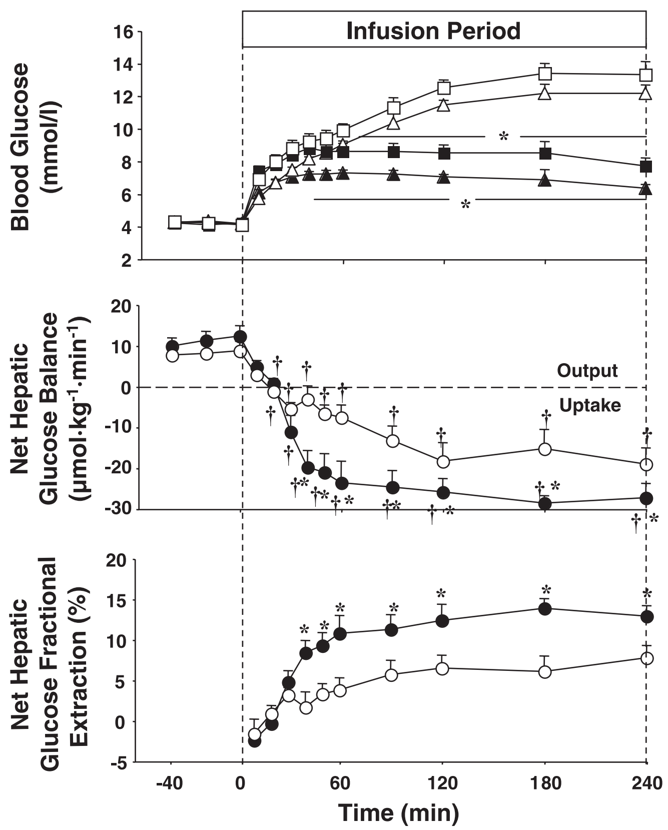

Fig. 2. Arterial (triangles) and portal (squares) plasma glucose levels, changes in net hepatic glucose balance, and hepatic fractional glucose extraction before and during continuous intraportal infusion of glucose at 44.4 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ with (filled symbols) and without (open symbols) continuous intraportal infusion of fructose at 2.22 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ in 42-h fasted conscious dogs. Data represent means ± SE, $n = 5$ for each group. †Significantly different from control period in identical vessel ($P < 0.05$). *Significantly different from the corresponding value in control group ($P < 0.05$).

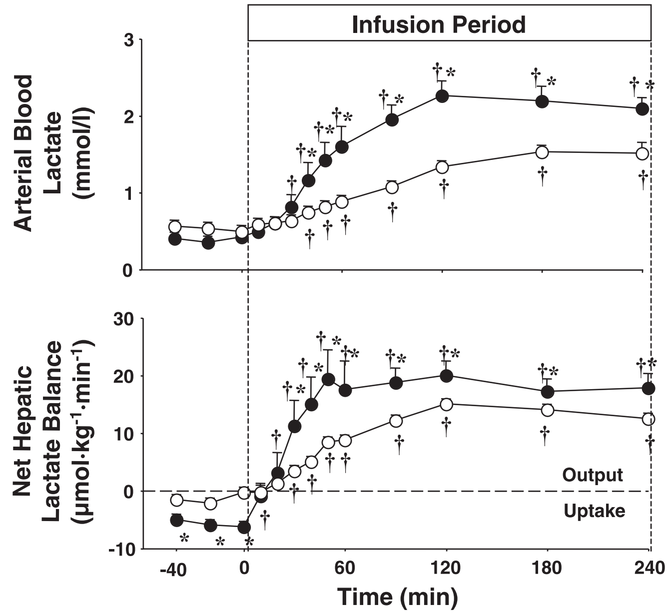

Fig. 3. Arterial blood lactate levels and net hepatic lactate balance before and during continuous intraportal infusion of glucose at 44.4 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ with (filled symbols) and without (open symbols) continuous intraportal infusion of fructose at 2.22 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ in 42-h fasted conscious dogs. Data represent means ± SE, $n = 5$ for each group. †Significantly different from control period in identical vessel ($P < 0.05$). *Significantly different from the corresponding value in control group ($P < 0.05$).

DISCUSSION

The results of the present study demonstrate that, even in the absence of an increase in plasma insulin, small amounts of fructose can significantly lessen the hyperglycemia resulting from intraportal glucose infusion by increasing in the ability of the liver to take up glucose and store it as glycogen.

In our previous study in 42-h-fasted conscious dogs (45), an increase in NHGU resulting from an intraportal infusion of a small amount of fructose was accompanied by increases in hepatic glucose 6-phosphate content, glycogen synthesis by the direct pathway, and glycolytic flux in the presence of the rise in plasma insulin levels, indicating that small amounts of fructose stimulate glucokinase flux in this condition. In the present study, in which plasma insulin levels were maintained at basal, fructose-stimulated increase in NHGU was also accompanied by increases in hepatic glucose 6-phosphate content, glycogen synthesis by the direct pathway, and glycolytic flux, suggesting that small amounts of fructose are able to

Table 2. *Arterial levels and NHB of blood alanine and glycerol and plasma NEFA levels before and during an intraportal infusion of glucose with and without continuous intraduodenal infusion of fructose in 42-h-fasted conscious dogs*

Group	Time, min						
		Infusion period					
	Control period	30	60	90	120	180	240
Alanine							
Control							
Blood levels	338±32	315±16	347±16	408±15	451±15	548±11	575±6
NHB	−2.3±0.3	−2.3±0.4	−2.1±0.3	−2.3±0.4	−2.3±0.3	−2.2±0.4	−2.2±0.5
Fructose							
Blood levels	282±27	322±42	424±57	554±58	660±35	760±46	818±62
NHB	−2.0±0.3	−1.5±0.3	−2.1±0.3	−1.8±0.4	−2.7±0.5	−3.1±0.6	−3.4±0.4
Glycerol							
Control							
Blood levels	55±7	56±7	46±4	53±6	46±5	43±8	45±7
NHB	−0.7±0.1	−1.0±0.2	−0.7±0.1	−1.1±0.4	−1.0±0.2	−1.0±0.3	−0.7±0.1
Fructose							
Blood levels	79±10	59±5	49±5	40±6	36±6	40±8	44±7
NHB	−1.3±0.1	−1.3±0.3	−1.0±0.3	−0.8±0.2	−0.6±0.2	−0.8±0.2	−0.9±0.1
NEFA							
Control							
Blood levels	564±50	553±80	502±90	423±76	406±91	334±62	392±56
NHB	−1.5±0.3	−1.8±0.2	−1.6±0.3	−0.2±0.6	−1.8±0.4	−1.6±0.4	−1.2±0.5
Fructose							
Blood levels	704±91	537±100	483±122	333±100	252±37	294±19	319±30
NHB	−1.6±0.7	−1.0±0.5	−1.2±0.6	−0.4±0.4	−0.5±0.3	−1.1±0.4	−0.9±0.4

Data are means ± SE; $n = 5$ dogs for each group. NEFA, nonesterified fatty acids; NHB, net hepatic balance. A negative value for NHB represents net uptake.

stimulate glucokinase flux even in the absence of a rise in plasma insulin. It has been demonstrated that fructose activates glucokinase activity in the liver via increasing intracellular concentration of fructose 1-phosphate (1, 19, 49). The conversion (phosphorylation) of fructose to fructose 1-phosphate is catalyzed by fructokinase, an enzyme not regulated by insulin (32). Indeed, the fractional extraction (50%) of fructose by the liver in the absence of the rise in plasma insulin (in the present study) is very similar with that (46%) in our previous study (45) in which small amounts of fructose were infused intraportally in the presence of hyperglycemia and hyperinsulinemia. Van Shaftingen et al. (49) and Agius and Peak (1) showed that the addition of very low concentrations of fructose rapidly increases fructose 1-phosphate content in cultured hepatocytes and induces the release of glucokinase from its regulatory protein even in the absence of insulin. Furthermore, it has been shown that in the absence of insulin, fructose at low extracellular concentrations (50–200 μmol/l) stimulated glucose phosphorylation as measured by the formation of ^3H_2O

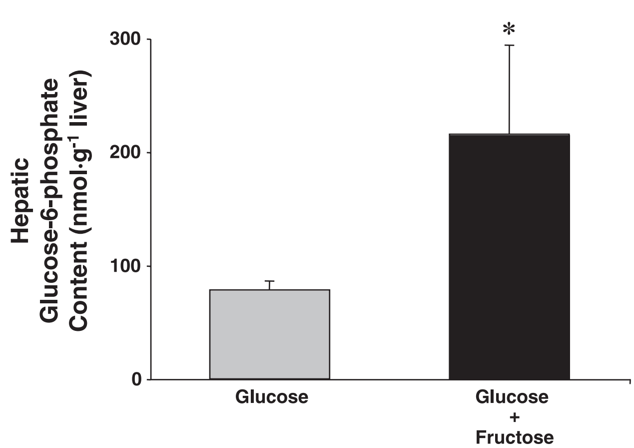

Fig. 4. Hepatic glucose 6-phosphate content at the end of experiment in 42-h-fasted conscious dogs. Data represent means ± SE; $n = 5$ for each group. *Significantly different from the corresponding value in control group ($P < 0.05$).

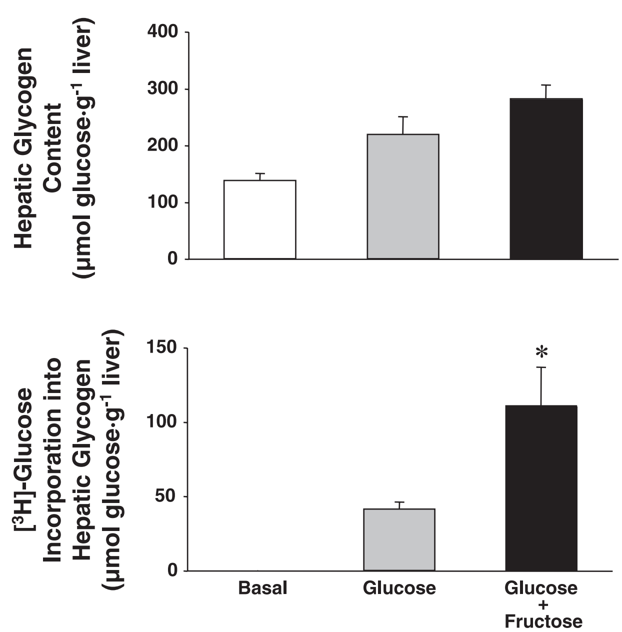

Fig. 5. Hepatic glycogen content and amount of glucose incorporated into glycogen by the direct pathway at the end of experiment in 42-h-fasted conscious dogs. Data represent means ± SE; $n = 5$ for each group. *Significantly different from the corresponding value in control group ($P < 0.05$).

from [2-^{3}H]glucose and the glycolytic flux as measured by the release of ^3H_2O from [3-^{3}H]glucose (20, 49). Therefore, the production of fructose 1-phosphate from fructose and activation of GK by fructose 1-phosphate do not depend on an increase in plasma insulin levels.

Hepatic glycogen synthesis and glycolysis. The stimulation of NHGU by small amounts of fructose was accompanied by increased glycogen content in the liver even in the absence of the rise in plasma insulin. Net glycogen deposition depends on the activities of glycogen synthase and phosphorylase. The activities of these enzymes are regulated not only via phosphorylation/dephosphorylation but also allosterically by some metabolic intermediates (6, 17, 19). The effects of fructose administration on phosphorylase activity are controversial. Bollen et al. (7) observed an activation of phosphorylase and an inhibition of phosphorylase phosphatase by fructose 1-phosphate in liver extracts. Kaufmann and Froesch (27) showed an inhibition of phosphorylase by fructose 1-phosphate. Ercan-Fang et al. (17) reported that phosphorylase was directly inhibited by high concentration of fructose 1-phosphate but not by physiological levels of the sugar. There is no evidence that glycogen synthase activity is regulated directly by changing fructose 1-phosphate content (concentration). On the other hand, the inclusion of small amounts of fructose increased the intracellular content of glucose 6-phosphate, which has been reported to be a potent inhibitor of phosphorylase activity (2) and activator of glycogen synthase (50). There is in vitro evidence that glucose 6-phosphate can stimulate dephosphorylation of phosphorylase-a by stimulating phosphorylase phosphatase (9) and inhibit phosphorylation of phosphorylase-b by inhibiting phosphorylase kinase (47) by a substrate-mediated mechanism. Recently, Aiston et al. (2) changed glucose 6-phosphate content in cultured hepatocytes by using three different approaches, involving incubation with substrates, overexpression of glucokinase, and inhibition of glucokinase with 5-thioglucose, and demonstrated that phosphorylase-a activity was decreased by increasing glucose 6-phosphate content.

An activation of hepatic glycogen synthase has been repeatedly observed with fructose administration in in vivo and in vitro studies (48). Niewoehner et al. (38) suggested that activation of glycogen synthase by the administration of relatively small fructose loads to intact animals is secondary to increased glucose 6-phosphate, a potent activator for glycogen synthase (50). Glucose 6-phosphate inhibits phosporylase-a which inhibits glycogen synthase phosphatase allosterically (2) as mentioned before. It is possible, therefore, that fructose activates glycogen synthase via an increase in the intracellular content of glucose 6-phosphate, which in turn results from the activation of glucokinase and an increase hepatic glucose uptake. Therefore, a small amount of fructose was able to stimulate glycogen synthesis via activation of glycogen synthase and inhibition of glycogen phosphorylase by increasing glucose 6-phosphate resulting from the activation of glucokinase even in the absence of the rise in plasma insulin.

Net hepatic lactate production induced by the intraportal glucose load was increased by intraportal infusion of small amount of fructose even when plasma insulin levels remained basal. Although glucose per se is known to stimulate glycolysis via increasing intracellular concentration of fructose 2,6-bisphosphate by dephosphorylating fructose 6-phosphate,2-kinase:fructose 2,6-bisphosphatase (40), plasma glucose levels during the intraportal glucose load were lower in the presence of fructose infusion, indicating that increased glycolytic flux in the presence of fructose might not be mediated by the rise in plasma glucose. The increased intracellular content of glucose 6-phosphate in all likelihood increased glycolytic flux by its mass action. In addition, it is known that pyruvate kinase and phosphofructokinase are key regulatory sites in glycolysis. Low-fructose loads when given to intact animals (41) or isolated hepatocytes (20) increased the intracellular content of fructose 2,6-bisphosphate, a potent activator of phosphofructokinase (25). This effect might be secondary to increased glucose 6-phosphate content because fructose-induced activation of glycolysis was not observed in rat hepatocytes incubated in the presence of mannoheptulase which inhibited glucokinase (20). In perfused liver, pyruvate kinase has been reported to be activated only by very high fructose loads (1–5 mmol/l) (16). Even in the absence of the rise of plasma insulin levels, therefore, the inclusion of fructose could increase glycolytic flux via an increase in the intracellular glucose 6-phosphate content secondary to the activation of glucokinase, perhaps with an associated increase in the activity of phosphofructokinase even when plasma insulin remains at the basal.

Insulin stimulates glucose 6-phosphate disposal to glycogen by activating glycogen synthase and inactivating glycogen phosphorylase in the liver. It has been reported that insulin stimulates glycolysis in the liver by increasing the level of fructose 2,6-bisphosphate and by modifying the activity ratio of pyruvate kinase (4, 25). These actions of insulin accelerate the rate of glucose 6-phosphate disposal. Furthermore, insulin has been reported to induce glucokinase translocation in cultured rat hepatocytes (1) and in vivo in normal rats (13), although its mechanism remains unknown. However, it remains to study whether the rise in insulin interacts with fructose to stimulate NHGU additively or synergistically.

Increases in plasma insulin and/or glucose concentrations, glucose delivery to the liver, and the arterial-portal glucose gradient increase the magnitude of NHGU (12). To evaluate the effects of fructose on NHGU and intracellular glucose metabolism, plasma insulin and/or glucose concentrations, therefore, these parameters have to be matched between the fructose and control groups. In the present study, the fructose group had lower sinusoidal glucose levels and glucose delivery to the liver during the test period as a result of higher NHGU with an equivalent rate of intraportal glucose infusion compared with control group. Plasma insulin levels were also lower in the fructose group than the control group. The difference in plasma insulin levels resulted from the rise in the hormone in the control group despite continuous infusion of somatostatin to inhibit endogenous secretion of the hormone. Markedly increased plasma glucose levels might overcome the inhibitory effect of somatostatin. Therefore, our evaluation based on the differences in NHGU and intracellular glucose metabolism between the fructose and control groups may underestimate the effects of fructose on NHGU and intracellular glucose metabolism.

The present study demonstrated that small amount of fructose could stimulate NHGU independently from the rise in plasma insulin. Patients with type 2 diabetes exhibit impaired suppression of hepatic glucose production and a defect in hepatic glucose uptake in response to the rise in plasma glucose and their livers are insulin resistant (18, 21, 37). The human liver possesses glucokinase and the regulatory protein (49), and the amount of hepatic glucokinase in patients with type 2 diabetes remains at ~50% of that in normal subjects (10). Therefore, it is possible that in humans with type 2 diabetes, as well as in dogs, small amounts of fructose can increase hepatic glucose uptake by activating glucokinase. In fact, a number of studies recently suggested that such is the case. 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 (24) and that fructose decreases the glucose and insulin responses to an oral glucose tolerance test (36). Therefore, the addition of small amounts of fructose to glucose loads may be useful in lowering postprandial hyperglycemia in diabetic subjects with hepatic insulin resistance by increasing translocation of the available glucokinase.

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