Effects of fructose on hepatic glucose metabolism in humans

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Dirlewanger, Mirjam, Philippe Schneiter, Eric Jéquier, and Luc Tappy. Effects of fructose on hepatic glucose metabolism in humans. Am J Physiol Endocrinol Metab 279: E907–E911, 2000.—Hepatic and extrahepatic insulin sensitivity was assessed in six healthy humans from the insulin infusion required to maintain an 8 mmol/l glucose concentration during hyperglycemic pancreatic clamp with or without infusion of 16.7 μmol·kg⁻¹·min⁻¹ fructose. Glucose rate of disappearance (GRd), net endogenous glucose production (NEGP), total glucose output (TGO), and glucose cycling (GC) were measured with [6,6-²H₂]- and [2-²H₁]glucose. Hepatic glycogen synthesis was estimated from uridine diphosphoglucose (UDPG) kinetics as assessed with [1,¹³C]galactose and acetaminophen. Fructose infusion increased insulin requirements 2.3-fold to maintain blood glucose. Fructose infusion doubled UDPG turnover, but there was no effect on TGO, GC, NEGP, or GRd under hyperglycemic pancreatic clamp protocol conditions. When insulin concentrations were matched during a second hyperglycemic pancreatic clamp protocol, fructose administration was associated with an 11.1 μmol·kg⁻¹·min⁻¹ increase in TGO, a 7.2 μmol·kg⁻¹·min⁻¹ increase in NEGP, a 2.2 μmol·kg⁻¹·min⁻¹ increase in GC, and a 7.8 μmol·kg⁻¹·min⁻¹ decrease in GRd (P < 0.05). These results indicate that fructose infusion induces hepatic and extrahepatic insulin resistance in humans.

endogenous glucose production; glucose cycling; glycogen synthesis; insulin resistance; liver

FRUCTOSE MAY REPRESENT as much as 8% of total energy intake in European and North American countries (17). Its metabolic effects, however, remain controversial. Because its initial metabolic steps do not require insulin (13) and because it produces little rise in glycemia (5), it has been considered a therapeutic adjunct in the diet of diabetic patients. It also elicits a more important increase in energy expenditure that has been suggested to be beneficial for obese diabetic and nonobese patients (18, 21). In contrast with these expected beneficial effects, increasing the amount of fructose in the diet has proven to have deleterious consequences as well. In diabetic and nondiabetic humans, fructose-rich diets have been associated with insulin resistance, impaired glucose tolerance, high blood pressure, and dyslipidemia (6, 10).

Endogenous glucose production plays a prominent role in fasting and postprandial glucose homeostasis (8, 20). After an overnight fast, the liver is responsible for the major portion of glucose release, whereas the kidney contributes a minor portion (19). The bulk of an oral or intravenous fructose load is taken up by liver cells, where it is largely converted into glucose (15). A substantial portion is also taken up by the kidney (2). Despite this marked stimulation of gluconeogenesis, administration of pure fructose does not increase glucose production and elicits only small rises in glycemia (16, 22). We previously proposed that stimulation of hepatic glycogen synthesis and inhibition of net glyco- genolysis by fructose were involved in maintaining a constant glucose production (22).

It remains unknown, however, whether fructose interferes with the inhibition of glucose production normally observed after a carbohydrate meal. Such inhibition occurs as the result of inhibitory effects of both hyperglycemia and hyperinsulinemia on glucose-producing pathways (4, 8). We therefore assessed in healthy human volunteers the effects of fructose infusion on hepatic insulin sensitivity in conditions of moderate hyperglycemia. For this purpose, we monitored the plasma insulin concentration required to maintain glycemia at 8 mmol/l during somatostatin and low physiological glucagon infusion with or without fructose infusion (hyperglycemic pancreatic clamp). Endogenous glucose production and the cycling between glucose and glucose 6-phosphate were measured with [6,6-²H₂]- and [2-²H₁]glucose infusions and were compared with those measured in another group of subjects studied at the same insulin concentration as those obtained during fructose infusion. To evaluate the effects of fructose on hepatic glycogen metabolism, hepatic uridine diphosphoglucose (UDPG) turnover was also measured by infusing [¹³C]galactose and monitoring urinary acetaminophen glucuronide enrichment (9).

METHODS

Ten healthy human volunteers (6 males, 4 females) were selected to participate in one to three experimental protocols. They had a mean age of 23.9 ± 1.0 yr, a mean weight of 71.5 ± 4.8 kg, and a mean height of 177.6 ± 4.6 cm. They were all in good physical health and did not take any medi-
cation at the time the experiments were performed. None had a family history of diabetes or obesity among first-degree relatives. The experimental protocols were approved by the ethical committee of Lausanne University School of Medicine, and volunteers provided informed, written consent.

General procedures. All experiments began between 0700 and 0800 after an overnight fast. On their arrival in the metabolic investigation laboratory, the subjects were weighed and measured. They took their places in beds in a semirecumbent position, and two venous cannulas were inserted, one in an antecubital vein for infusion of hormones, tracers, and glucose and the other in a wrist vein of the contralateral arm for collection of blood samples. This hand was placed in a thermostabilized box heated at 50°C to achieve partial arterialization of venous blood.

Protocol 1: hyperglycemic pancreatic clamp without fructose infusion. Six subjects (4 males, 2 females) took part in this protocol. Cyclic somatostatin (Stilastatin; Serono, Aubonne, Switzerland) was continuously infused at a rate of 350 μg/h. Glucagon (Glucagone; Novo Nordisk, Copenhagen, Denmark) was infused at a rate of 0.5 ng·kg⁻¹·min⁻¹ during 180 min (time 0-minute 180) to obtain low physiological glucagon concentrations. An infusion of crystalline insulin (Actrapid HM; Novo Nordisk) was adjusted during the initial 90 min of the protocol to achieve steady plasma glucose concentrations of ~8 mmol/l. Primed (1.6 μmol/kg) continuous (0.016 μmol·kg⁻¹·min⁻¹) infusions of [2-²H₁]glucose and [6,6-²H₂]glucose (both from Masstrace, Worcester, MA) were administered throughout the experiment to measure the rates of glucose production and glucose cycling. [1-¹³C]galactose (Cambridge Isotope Laboratory, Cambridge, MA) was continuously infused at a rate of 1.5 μg·kg⁻¹·min⁻¹ throughout the whole experiment. Oral acetaminophen (Tylenol, Janssen-Cilag, Baan, CH, Switzerland) was administered as 200-mg doses at time −30 and at 0, 60, and 120 min. Blood samples were taken at 5- to 10-min intervals to check plasma glucose by use of a glucose analyzer II (Beckman, Fullerton, CA) and at 30-min intervals for determination of hormones, substrates, and tracer concentrations. Two urine collections were performed, one, which was discarded, between 0 and 120 min and the second one between 120 and 180 min. This latter collection was used for purification of acetaminophen glucuronide.

Protocol 2: hyperglycemic pancreatic clamp with fructose infusion. Six subjects (4 males, 2 females) took part in this protocol. The experiment was identical to protocol 1, but subjects were in addition continuously infused with 16.7 μmol·kg⁻¹·min⁻¹ fructose. The infusion rate of insulin was adjusted during the initial 90 min of the protocol to achieve steady plasma glucose concentration of ~8 mmol/l.

Protocol 3: hyperglycemic-hyperinsulinemic clamp studies. Seven subjects (4 males, 3 females) took part in this protocol. Somatostatin and glucagon were infused as in protocols 1 and 2, while crystalline insulin was administered at a rate of 1.5 pmol·kg⁻¹·min⁻¹ to reproduce the plasma insulin concentrations obtained in protocol 2. An infusion of 20% dextrose was periodically adjusted to maintain plasma glucose at 8 mmol/l. The exogenous glucose was labeled with 1.5% [6,6-²H₂]glucose and [2-²H₁]glucose to minimize changes in plasma glucose enrichments over time.

Analytical procedures. Plasma [6,6-²H₂]- and [2-²H₁]glucose were determined by gas chromatography-mass spectrometry (Hewlett Packard, Palo Alto, CA) as previously described. Plasma insulin (Biodata, Guidonia Montecello, Italy) and glucagon (Linco Research, St. Charles, MO) were measured by radioimmunoassay using commercial kits.

Urinary acetaminophen-glucuronide was purified, and glucuronide was converted into glucose as described by Magnussen et al. (12). To determine ¹³C abundance of glucuronide, glucose obtained with this procedure was purified, and its ¹³C enrichment was measured.

Calculations. Glucose rates of appearance (GRₐ) and disappearance (GRₜ) were calculated with Steele’s equations for steady-state conditions in protocols 1 and 2, and “hot infusate” equations in protocol 3 (7, 25). Total glucose output (TGO) was obtained from [2-²H₁]glucose enrichment and net endogenous glucose production (NEGP) from [6,6-²H₂]glucose enrichments. In protocol 3, the exogenous glucose infusion was subtracted from GRₜ. Glucose cycling was calculated as the difference between TGO and NEGP (24). Hepatic UDPG turnover was calculated as [1-¹³C]galactose infusion/¹³C enrichment of urinary glucuronide.

![Fig. 1. Plasma, glucose, insulin, and glucagon concentrations during hyperglycemic pancreatic clamp without fructose (F−), with fructose infusion (F+), and in hyperglycemic hyperinsulinemic clamp studies (HI). *P < 0.05 vs. F−.](image)
Statistical analysis. All measurements performed during the last 60 min of the protocol were averaged for data presentation and statistical analysis. Results in the text, figures, and tables are shown as means ± SE unless otherwise stated. Between protocols, comparisons were performed by paired (protocol 1 vs. protocol 2) or unpaired (protocol 3 vs. protocols 1 and 2) t-tests.

RESULTS

In the three protocols, all measured parameters reached a plateau during the 3rd hour. Values obtained during this period were averaged for presentation and statistical analysis.

Hormone and substrate concentrations. Plasma glucose concentrations were 7.9 ± 0.3, 7.9 ± 0.9, and 9 ± 0.2 mmol/l in protocols 1, 2, and 3, respectively (Fig. 1). The insulin infusion required to maintain glycemia at ~8 mmol/l was, however, increased 133% during fructose infusion (from 0.63 ± 0.16 without fructose to 1.47 ± 0.3 pmol·kg⁻¹·min⁻¹ with fructose, P < 0.01). As a consequence, plasma insulin concentrations (110 ± 20 pmol/l) were 64% higher compared with the protocol without fructose infusion (67 ± 7, P < 0.01). In hyperinsulinemic clamps, infusion of 1.5 pmol·kg⁻¹·min⁻¹ insulin resulted in plasma insulin concentrations (122 ± 11) in the same range as those obtained during the fructose experiment.

Glucose production and glucose cycling. Steady-state plasma [6,6-²H₂]- and [2-²H₁]glucose enrichments are shown in Fig. 2. During fructose infusion, NEGP, TGO, and GC were similar to those observed in protocol 1 without fructose infusion. NEGP was, however, 174% higher (P < 0.05), TGO 199% higher (P < 0.05), and GC 133% higher (P < 0.05) than values obtained in the presence of the same hyperinsulinemia without fructose infusion in protocol 3 (Fig. 3). GR₆₅ was identical during protocols 1 and 2, i.e., with or without fructose. With fructose, however, it was reduced by 37% compared with values measured at similar insulinemia in protocol 3 (P < 0.05).

UDPG kinetics. UDPG turnover was increased by 99% during fructose infusion compared with control experiments without fructose (P < 0.01; Fig. 4). During hyperinsulinemic hyperglycemic clamp studies, which increased plasma insulin concentrations to levels similar to those attained during fructose studies, UDPG turnover was not superior to control experiments without fructose (Fig. 4).

DISCUSSION

The major result of this study indicates that fructose infusion induced major alterations in the regulation of endogenous glucose production. This conclusion is supported by two observations. First, insulin infusion
rates and plasma insulin concentrations required to maintain glycemia at 8 mmol/l in the presence of fixed, low physiological glucagonemia were increased by 133% during fructose infusion compared with control experiments with no fructose infusion. Second, the endogenous glucose production measured during fructose infusion was increased 2.7-fold compared with that observed during a hyperinsulinemic clamp at similar insulin and glucagon concentrations.

TGO and GC were determined with the simultaneous use of [6,6-2H2]- and [2-2H1]glucose as tracers (24). Both values were similar in protocols 1 and 2. However, in protocol 2 (fructose infusion), an increased insulin infusion rate was needed to maintain the mild hyperglycemia. This led us to study another group of subjects in a third protocol. In this protocol, somatostatin, insulin, and glucagon were all infused at the same rate as during glucose infusion while glucose was infused to obtain a comparable glucose concentration. TGO and GC were both markedly reduced under these conditions. Because insulin, glucagon, and glucose concentrations were similar in this third protocol (hyperinsulinemic clamp) and in protocol 2 (fructose infusion), the changes in glucose kinetics can be unequivocally attributed to fructose infusion. This demonstrates that fructose, at similar insulinemia, stimulates these two processes.

Increased TGO indicates that the absolute rate of glucose 6-phosphate hydrolysis and release of free glucose from liver cells was increased during fructose infusion. Simultaneously, GC was increased, indicating enhanced reuptake and phosphorylation of glucose by liver cells. GC results from the simultaneous activities of hepatic glucokinase and glucose-6-phosphatase. Under conditions of net hepatic glucose release, as during the present experiments, GC is essentially dependent on glucokinase activity. In vitro and in vivo experiments have documented that glucokinase activity is limited by its binding to a regulatory protein and that fructose 1-phosphate increases glucokinase activity by allowing dissociation of inactive bound glucokinase, thus resulting in increased amounts of unbound, active glucokinase in the cytosol (1, 14, 23). This mechanism constitutes a plausible explanation for the stimulation by fructose of GC. Furthermore, stimulation of GC during fructose administration may constitute a regulatory mechanism to prevent excessive increases in net glucose output. A similar mechanism has been proposed to be operative during dexamethasone treatment in healthy humans (24).

In these experiments, [1,13C]galactose was administered to label intrahepatic UDPG, whereas acetalaminophen was used to noninvasively sample intrahepatic UDPG pool. This procedure, aimed at assessing intrahepatic UDPG kinetics and hepatic glycogen synthesis, has been extensively discussed by Hellerstein et al. (9). Our data indicate that fructose markedly increased intrahepatic UDPG turnover and thus stimulated hepatic glycogen synthesis. This observation is entirely consistent with previous experiments that documented a large increase in hepatic glycogen stores after fructose administration (15). It results directly from intrahepatic fructose metabolism, because it was not observed during the hyperinsulinemic clamp studies.

In addition to its effect on hepatic metabolism, fructose infusion also produced extrahepatic insulin resistance, as indicated by a substantial reduction in GR4 compared with hyperinsulinemic clamp studies. The mechanisms responsible for inhibition of extrahepatic insulin-mediated glucose disposal remain, however, unknown. It is unlikely to involve fructose metabolism in skeletal muscle, because it represents only a minor portion of glucose metabolism (2). It can be speculated that this effect may be related to intermediary metabolites produced during hepatic fructose metabolism.

In conclusion, our results indicate that acute fructose infusion induces both extrahepatic and hepatic insulin resistance, the latter presumably being secondary to an increased intrahepatic glucose 6-phosphate synthesis. It remains to be determined whether fructose ingested as part of the diet exerts the same effects. These results also raise the question whether the increased extrahepatic endogenous gluconeogenic substrate production known to occur in type 2 diabetes (3, 11) induces hepatic insulin resistance through similar mechanisms.

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REFERENCES


