Supplemental Fructose Attenuates Postprandial Glycemia in Zucker Fatty fa/fa Rats

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ABSTRACT Experiments were conducted to evaluate the effects of supplemental fructose on postprandial glycemia. After overnight food deprivation, Zucker fatty fa/fa rats were given a meal glucose tolerance test. Plasma glucose response was determined for 180 min postprandially. At a dose of 0.16 g/kg body, fructose reduced (P < 0.05) the incremental area under the curve (AUC) by 34% when supplemented to a glucose challenge and by 32% when supplemented to a maltodextrin (a rapidly digested starch) challenge. Similarly, sucrose reduced (P = 0.0575) the incremental AUC for plasma glucose when rats were challenged with maltodextrin. Second-meal glycemic response was not affected by fructose supplementation to the first meal, and fructose supplementation to the second meal reduced (P < 0.05) postprandial glycemia when fructose had been supplemented to the first meal. In a dose-response study (0.1, 0.2, and 0.5 g/kg body), supplemental fructose reduced (P < 0.01) the peak rise in plasma glucose (linear and quadratic effects). In the final experiment, a low dose of fructose (0.075 g/kg body) reduced (P < 0.05) the incremental AUC by 18%. These data support the hypothesis that small amounts of oral fructose or sucrose may be useful in lowering the postprandial blood glucose response. J. Nutr. 132: 1219–1223, 2002.

KEY WORDS: • fructose • glycemia • Zucker fatty fa/fa rats • blood glucose control

In the evaluation of a novel acid-induced viscosity agent on postprandial glycemia, which delivered ~61 g of glucose, Wolf (1) observed that the peak serum glucose response was lower (6.8 ± 0.19 and 6.5 ± 0.23 mmol/L for control and acid-induced viscosity test meals, respectively) than predicted from published literature (2–5). For example, Crapo et al. (3) found a peak postprandial serum glucose response of ~9.2 mmol/L in normal (nondiabetic) adult subjects given a 50-g load of dextrose. This discrepancy led to a hypothesis that supplemental fructose, which was present in the glucose-based experimental beverages at ~12 g, attenuates postprandial glycemia.

Fructose alone increases postprandial blood glucose concentrations less than isoenergetic amounts of glucose (3,6,7). Shiota et al. (8) found that intraportal infusion of small amounts of fructose augments net hepatic glucose uptake during hyperglycemic hyperinsulinemia in dogs. In healthy humans, Petersen et al. (9) observed that fructose infusion resulted in a threefold increase in net hepatic glycogen synthesis during euglycemic hyperinsulinemia. In isolated rat hepatocytes, Fillat et al. (10) demonstrated that fructose at low concentrations stimulated the glycolytic flux. This effect may be mediated through the control of hepatic glucose phosphorylation. Glucokinase is acutely regulated by fructose-6-phosphate and fructose-1-phosphate, two metabolites whose effects are dependent upon an inhibitory protein that tightly binds to glucokinase (11). Fructose-6-phosphate promotes, but fructose-1-phosphate inhibits binding of the inhibitory protein to glucokinase. Thus, dietary fructose may promote hepatic glucose utilization by an indirect mechanism. Fructose is converted in the liver directly to fructose-1-phosphate (via fructokinase, an enzyme present only in the liver), which competes with fructose-6-phosphate on the glucokinase regulatory protein and activates glucokinase by promoting dissociation of its inhibitory protein. Supplemental dietary fructose may enhance glucose flux through glucokinase in people with type 2 diabetes mellitus; they have an impaired ability to suppress endogenous glucose production during hyperglycemia, due in part to decreased glucose-induced flux through glucokinase (12). With an improvement in postprandial hepatic glucose uptake, the blood glucose level may be reduced after a meal containing supplemental fructose. Wolf (1) postulated that fructose supplementation to a glucose challenge attenuates the glycemic response in healthy nondiabetic adult subjects.

A series of rat experiments were conducted to evaluate the effects of supplemental fructose on postprandial glycemia. In Zucker fatty fa/fa rats (a model of type 2 diabetes mellitus), we found that supplemental fructose attenuates postprandial glycemia.

MATERIALS AND METHODS

Animals. Experiments were performed on 400- to 450-g Zucker fatty fa/fa rats (Harlan Sprague Dawley, Indianapolis, IN). Rats were...
individually housed in microisolator cages on dry bedding and were given free access to water and nonpurified rat diet (pelleted; 8640 Harlan Teklad 22/5 Rodent Diet; Harlan Teklad, Madison, WI). According to the manufacturer, the average nutrient composition (g/100 g) of the nonpurified diet was: protein, 22.58; fat, 5.23; fiber, 3.94; ash, 7.06; and nitrogen-free extract, 51.19. The housing facility was maintained at 19–23°C, 30–70% relative humidity, with a 12-h light:dark cycle. Rats were handled 4 to 5 times per week and were trained to consume test meals orally for the meal glucose tolerance test (MGTT).³ The animal use protocol was reviewed and approved by The Ohio State University Animal Care Committee (Columbus, OH).

**Experimental design.** In a series of experiments, dietary treatments were evaluated in a randomized crossover design with a 7-d (range 6–9 d) washout period between each MGTT. Within each experiment, every rat received each treatment. Rats were routinely fed the nonpurified diet. After overnight food deprivation, rats were orally fed test meals as a solution. Rats consumed the test meal within 1 min. Blood samples were collected from the tail vein and immediately analyzed for plasma glucose by the glucose oxidase method utilizing a Precision® G Blood Glucose Testing System (Medisense, Bedford, MA) before (0 min) and 30, 60, 90, 120 and 180 min postprandially. Rats had free access to water throughout the MGTT.

**Test carbohydrates.** Raw cornstarch (RCS; Argo, CPC International, Englewood Cliffs, NJ) was obtained from a local grocery. Purified glucose, fructose, sucrose and maltose were obtained from Sigma Chemical (St. Louis, MO). Lodex 15 (maltodextrin) was obtained from Cerestar USA (Hammond, IN) and had a dextrose equivalence of 15.

**Experiment 1.** The objective of this experiment was to compare a high and low glycemic carbohydrate in the Zucker fatty fafa model of type 2 diabetes mellitus. The postprandial glycemic response to glucose was compared with a slowly digested starch, RCS (13–15) in 20 male rats. Two dietary treatments were evaluated, i.e., glucose and RCS. Carbohydrates were made in 500 mL solutions with water before the challenge. Test meal volume was ~1 mL and was adjusted such that each rat was delivered an equivalent carbohydrate challenge (1.0 g/kg body).

**Experiment 2.** The objective of this experiment was to evaluate the effects of supplemental fructose on the postprandial glycemic response to glucose in 10 female Zucker fatty fafa rats. Two dietary treatments were evaluated, i.e., glucose (1.0 g/kg body) alone and glucose plus supplemental fructose (0.16 g/kg body). This fructose dose approximates that fed previously by Wolf (1).

**Experiment 3.** The objective of this experiment was to evaluate the effects of supplemental fructose on the postprandial glycemic response to a rapidly digested starch. Maltodextrin (partially hydrolyzed cornstarch) was chosen as the rapidly digested starch (16). Two dietary treatments were evaluated, i.e., maltodextrin (1.0 g/kg body) alone and maltodextrin plus supplemental fructose (0.16 g/kg body). Maltodextrin was made into a 500 g/L solution with water, and fructose was added to the appropriate treatment. Each solution was heated in a microwave for 30 s to completely solubilize the carbohydrate solutions 1 h before testing in 10 male Zucker fatty fafa rats.

**Experiment 4.** The objective of this experiment was to evaluate whether supplemental sucrose (as an indirect source of fructose) would have the same effect as purified fructose on the postprandial glycemic response to a rapidly digested starch. Two dietary treatments were evaluated, i.e., maltodextrin (1.0 g/kg body) plus maltose (0.16 g/kg body) and maltodextrin (1.0 g/kg body) plus sucrose (0.32 g/kg body, which is 0.16 g/kg body sucrose equivalent). Treatments were prepared as described in Experiment 3 and fed to 10 male Zucker fatty fafa rats. One rat did not complete the sucrose treatment because food had not been withheld overnight.

**Experiment 5.** The objective of this experiment was to evaluate the effects of supplemental fructose on second meal glucose tolerance. The two test meals were maltodextrin (1.0 g/kg body) alone (meal 1) and maltodextrin plus supplemental fructose (0.16 g/kg body) (meal 2). A 3-h MGTT was conducted in the morning followed ~1.5 h later (i.e., 4.5 h after the start of the first MGTT) by a second 3-h MGTT. The four treatments were as follows: 1) meal 1 followed by meal 1 (M/M); 2) meal 1 followed by meal 2 (M/F); 3) meal 2 followed by meal 1 (F/M); and 4) meal 2 followed by meal 2 (F/F). Treatments were prepared as described in Experiment 3 and fed to 20 male Zucker fatty fafa rats. Many data points were missing at the 180-min time point; therefore, this time point was dropped from the analysis.

**Experiment 6.** The objective of this experiment was to evaluate the dose response of supplemental fructose. Four dietary treatments were evaluated, i.e., maltodextrin (1.0 g/kg body) alone and maltodextrin plus supplemental fructose at 0.10, 0.20 or 0.50 g/kg body. Treatments were prepared as described in Experiment 3 and fed to 20 male Zucker fatty fafa rats.

**Experiment 7.** The objective of this experiment was to evaluate a low dose of supplemental fructose. Two dietary treatments were evaluated, i.e., maltodextrin (1.0 g/kg body) alone and maltodextrin plus supplemental fructose (0.075 g/kg body). Treatments were prepared as described in Experiment 3 and fed to 19 male Zucker fatty fafa rats.

**Calculations and statistics.** A positive incremental area under the glucose curve (AUC) over the 180-min postprandial period was calculated according to Wolter et al. (17). If a rat had one or more glucose measurements missing between 0 and 180 min (both inclusive) during a MGTT, its data for that MGTT were not included in the analyses of peak incremental change from baseline (i.e., peak rise) and positive incremental AUC. Data were analyzed using a mixed model for crossover trials, with treatment and period effects as fixed, and rat effect as random. Baseline blood glucose concentration was used as a covariate for blood glucose concentrations at individual postprandial time points. Different covariance structures were tested according to Brown and Prescott (18). Model fit was checked by comparing Akaike’s Information Criterion. Composition symmetry variance pattern was found to be the most adequate for all experiments. Residuals from these models were plotted against the predicted values to verify the appropriateness of the model. In Experiment 5, treatments were arranged as a 2 × 2 factorial by the presence or absence of fructose at the first or second meal. The first meal fructose × second meal fructose interaction was not significant. In Experiment 6, the linear and quadratic effects of fructose dose were examined. Differences were considered significant if α was < 0.05; P-values reported are two-sided (SAS version 8.0, SAS Institute, Cary, NC).

**RESULTS AND DISCUSSION**

In the Zucker fatty fafa rat model of type 2 diabetes mellitus, we evaluated a low (RCS) and high glycemic test meal (glucose). The postprandial glycemic response to RCS was delayed and the early phase excursion reduced (P < 0.01) compared with a glucose challenge (Fig. 1). Peak rise in plasma glucose was lower (P < 0.01) for RCS (5.03 ± 0.38 vs. 6.32 ± 0.38 mmol/L). These in vivo rat data are consistent with the starch hydrolysis data of Wolf et al. (15), who found that RCS had a slow rate of in vitro hydrolysis, and the in vivo human findings of Wolf (1). Furthermore, it corroborates the use of RCS as nutritional therapy for the prevention of nighttime hypoglycemia (14,19,20). Using the Zucker fatty fafa rat model, we were able to differentiate a low and high glycemic test meal.

Shiota et al. (8) found that intraportal infusion of small amounts of fructose augments net hepatic glucose uptake during hyperglycemic hyperinsulinemia in dogs. In the present study, orally supplemented fructose attenuated the postprandial glycemic response to glucose (Fig. 2) and to a rapidly digested starch (Fig. 3). At a dose of 0.16 g/kg body, fructose

³ Abbreviations used: AUC, area under the curve; F/F, fructose-supplemented test meal followed by fructose-supplemented test meal; F/M, fructose-supplemented test meal followed by maltodextrin test meal; M/F, maltodextrin test meal followed by fructose-supplemented test meal; MGTT, meal glucose tolerance test; M/M, maltodextrin test meal followed by maltodextrin test meal; RCS, raw cornstarch.
reduced \((P < 0.05)\) the incremental AUC by 34% when supplemented to a glucose challenge and by 32% when supplemented to a maltodextrin challenge. In addition, the peak rise in plasma glucose was lower \((P < 0.05)\) when fructose was supplemented to a glucose (2.45 ± 0.35 vs. 3.80 ± 0.35 mmol/L; Glucose vs. RCS) challenge. The present study supports the hypothesis that orally administered supplemental fructose attenuates postprandial glycemia.

Sucrose (glucose \(\alpha\)-1,2 fructose) has a reduced glycemic index (6). Because sucrose is well absorbed in the small intestine (21–23), we postulated that sucrose, as an indirect source of fructose, would reduce postprandial glycemia compared with maltodextrin alone. Similar to our results with fructose, sucrose tended to reduce \((P = 0.0575)\) the incremental AUC for plasma glucose (Fig. 4). The peak rise in plasma glucose was lower \((P < 0.01)\) when rats were fed supplemental sucrose (5.43 ± 0.58 vs. 7.12 ± 0.56 mmol/L). Small amounts of oral sucrose reduced postprandial hyperglycemia in Zucker fatty \(fa/fa\) rats.
As previously noted, Shiota et al. (8) found that intraportal infusion of small amounts of fructose augment net hepatic glucose uptake. In addition, they determined that 69% of this glucose was stored as hepatic glycogen. Thus, we asked the question, can liver glycogen stores be maximized and worsen postprandial glycemia at the second meal? This question was addressed by evaluating the effects of supplemental fructose at the second meal. Treatments were arranged as a $2 \times 2$ factorial by the presence or absence of fructose at the first or second meal. Second-meal glycemic response was not affected by fructose supplementation to the first meal, and fructose supplementation to the second meal reduced ($P < 0.05$) postprandial glycemia when fructose had been supplemented to the first meal (Fig. 5). These results suggest that glycogen stores may not be filled or that fructose enhances glycolysis.

The latter idea is supported by the study of Atkinson et al. (24), who found that fructose feeding raised plasma lactate concentration in rats. Further research is required in this area to understand the metabolic implications of fructose supplementation.

The dose-related effect of supplemental fructose on an oral glucose tolerance test in AP rats was recently reported in abstract form (24). These investigators found that high levels of fructose supplementation (1, 2 and 4 g/kg body) reduced the early (10 min) postprandial plasma glucose concentrations after a glucose challenge. In the present study, we evaluated the dose response of fructose supplementation at lower levels (0.1, 0.2 and 0.5 g/kg body). Similar to the findings of Atkinson et al. (24), all three doses reduced ($P < 0.05$) the early rise in plasma glucose; however, incremental AUC for plasma glucose was unaffected (Fig. 6). Supplemental fructose reduced ($P < 0.01$) the peak rise in plasma glucose (linear and quadratic effects). A final experiment was conducted to test an even lower supplemental fructose dose (0.075 g/kg body). Again, fructose reduced ($P < 0.05$) the postprandial glycemic response (Fig. 7). The incremental AUC for plasma glucose was reduced ($P < 0.05$) 18%. The peak rise in plasma glucose was lower ($P < 0.01$) when rats were fed supplemental fructose (4.99 ± 0.27 vs. 6.34 ± 0.27 mmol/L). These findings have great clinical potential for the prevention of postprandial hyperglycemia in people with diabetes. Because fructose and sucrose intake have been shown to cause hypertriacylglycerolemia and hypercholesterolemia in rats (25) and humans (26–28), nutritional recommendations have been made to avoid supplementation of simple sugars to the diabetic diet (29,30). However, these negative attributes of fructose feeding need to be further explored.

**FIGURE 5** Second-meal postprandial glycemic response and incremental area under the curve (AUC) after a 1.0 g/kg body oral maltodextrin (Maltodextrin) or the same plus 0.1, 0.2 or 0.5 g/kg body supplemental fructose (Maltodextrin + fructose) challenge in male Zucker fatty fa/fa rats (Experiment 5). The four treatments were: 1) M followed by M (M/M); 2) M followed by F (M/F); 3) M followed by M (F/M); and 4) M followed by F (F/F).

**FIGURE 6** Postprandial glycemic response and incremental area under the glucose curve (AUC) after a 1.0 g/kg body oral maltodextrin (Maltodextrin) or the same plus 0.1, 0.2 or 0.5 g/kg body supplemental fructose (+0.1 fructose, +0.2 fructose and +0.5 fructose, respectively) challenge in male Zucker fatty fa/fa rats (Experiment 6). Basal fasting plasma glucose concentrations were not different (5.65 ± 0.20, 5.60 ± 0.19, 5.65 ± 0.19 and 5.40 ± 0.19 mmol/L for Maltodextrin, +0.1 fructose, +0.2 fructose and +0.5 fructose, respectively). Data are least squares means ± SEM, n = 20, except for +0.1 fructose n = 19. Linear effect of fructose, *$P < 0.05$. Quadratic effect of fructose, **$P < 0.05$. AUC, area under the curve; SEM, standard error of the mean; $n$, number of samples.
are well documented only at high (>17% total energy) dietary intakes (27,31). The findings of the present study support a low level of dietary supplementation of fructose for the attenuation of postprandial glycemia.

In conclusion, these data support the hypothesis that small amounts of oral fructose or sucrose may be useful in lowering the postprandial blood glucose response. These findings provide further support for the addition of fruit and honey, which naturally contain fructose and sucrose, to the diabetic diet. This concept deserves further clinical evaluation because it may be useful for the dietary treatment of people with diabetes.

LITERATURE CITED