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Acute Fructose Administration Decreases the Glycemic Response to an Oral Glucose Tolerance Test in Normal Adults*

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ABSTRACT

In animal models, a small (catalytic) dose of fructose administered with glucose decreases the glycemic response to the glucose load. Therefore, we examined the effect of fructose on glucose tolerance in 11 healthy human volunteers (5 men and 6 women). Each subject underwent an oral glucose tolerance test (OGTT) on 2 separate occasions, at least 1 week apart. Each OGTT consisted of 75 g glucose with or without 7.5 g fructose (OGTT+F or OGTT-F), in random order. Arterialized blood samples were obtained from a heated dorsal hand vein twice before ingestion of the carbohydrate and every 15 min for 2 h afterward. The area under the curve (AUC) of the change in plasma glucose was 19% less in OGTT+F *vs.* OGTT-F ($P < 0.05$). Glucose tolerance was improved by fructose in 9 subjects and worsened in 2. All 6 subjects with the largest glucose AUC during OGTT-F had a decreased response during OGTT+F (31 ± 5% decrease). The insulin AUC did not differ between the 2 studies. Of the 9 subjects with improved glucose tolerance during the OGTT+F, 5 had smaller insulin AUC during the OGTT+F than the OGTT-F. Plasma glucagon concentrations declined similarly during OGTT-F and OGTT+F. The blood lactate response was about 50% greater during the OGTT+F ($P < 0.05$). Neither nonesterified fatty acid nor triglyceride concentrations differed between the two OGTT. In conclusion, low dose fructose improves the glycemic response to an oral glucose load in normal adults without significantly enhancing the insulin or triglyceride response. Fructose appears most effective in those normal individuals who have the poorest glucose tolerance. (*J Clin Endocrinol Metab* **85:** 4515–4519, 2000)

ACUTE AND CHRONIC glycemic benefits of fructose as a substitute for other carbohydrates in both normal and diabetic humans have been recognized for some time. Crapo *et al.* (1) studied the glucose and insulin responses of normal subjects, individuals with glucose intolerance, and subjects with noninsulin-dependent (type 2) diabetes during the 3-h period after the ingestion of 50 g carbohydrate in the form of glucose, fructose, or sucrose, either alone or in conjunction with protein and fat. In all three groups of subjects, the glycemic responses were smallest when fructose was ingested, whether alone or as a part of a meal containing protein and fat. In the normal subjects and those with glucose intolerance, the postingestion insulin concentrations were also lower in response to ingestion of fructose than to ingestion of sucrose or glucose. In some investigations, chronic use of large amounts of fructose (~20% of total energy intake) to replace other carbohydrates in the diet has improved metabolic control in individuals with diabetes, whereas other investigations have found little glycemic benefit from the use of fructose (for reviews, see Refs. 2 and 3). Even where improvements in glycemic control have been observed, however, these benefits were offset by increases in total and low density lipoprotein cholesterol and/or triglycerides in susceptible individuals (4). Hypertension and insulin resistance have also been observed (2). Therefore, the use of large amounts of fructose in the diet on a daily basis may not be desirable. Evidence from animal models suggests, however, that glycemic benefits may occur even with the ingestion of very small amounts of fructose.

In conscious dogs receiving an intraduodenal glucose infusion at 8 mg/kg·min, with or without the addition of fructose to the infusate at 0.4 mg/kg·min, the net hepatic fractional glucose extraction was 2-fold greater during the infusion of fructose than during the delivery of glucose alone (5). The increase in the arterial blood glucose concentration during fructose infusion was only half as great as it was in the absence of fructose. Moreover, the enhancement of the liver's role in glucose disposal and the reduction in the glycemic response were not due to an increase in the insulin response, because the arterial plasma insulin concentration was only about half as high during fructose infusion as it was during the infusion of glucose alone (5).

The effect of catalytic amounts of fructose on glucose tolerance in humans is unknown. Moreover, the effect of small amounts of fructose on serum lipids has not been examined. Therefore, the current study was carried out to determine whether the addition of a small amount of fructose to a standard 75-g glucose tolerance test would reduce the glycemic response in normal humans without adversely affect-

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ing circulating triglyceride concentrations (the earliest lipid abnormality observed with ingestion of large amounts of fructose).

Subjects and Methods

Subjects

Studies were conducted on 11 healthy volunteers (5 men and 6 women; 2 African-American, 3 Asian, and 6 Caucasian; age, 29 ± 2 yr; body mass index, 23.6 ± 0.9 kg/m^2). Hemoglobin A_{1c} in these subjects ranged from 4.5–6.4% (normal, 4–6.5%). The subjects were taking no regular medications, and they had normal blood counts, serum electrolytes, and liver and renal function. None of them had first degree relatives with diabetes. All subjects consumed a diet containing at least 200 g carbohydrate daily for a week before study. The studies were approved by the institutional review board of Vanderbilt University Medical Center, and all subjects gave written informed consent before study.

Experimental design

All subjects were studied twice in a single blind, randomized fashion, with the two studies in the same subject 13 ± 2 days apart. The subjects were admitted to the General Clinical Research Center of Vanderbilt University Medical Center the evening before each study and were studied after a 10-h overnight fast. At approximately 0800 h on the day of study, a 20-gauge iv cannula was inserted retrograde into a dorsal vein in one hand. The hand was placed in a thermostatically controlled warmed box, where it remained throughout the study so that arterialized venous blood samples could be obtained (6).

Two basal blood samples, 15 min apart, were drawn before the start of each study. After the second sample was drawn, the subject rapidly (within 1 min) drank a solution containing 75 g glucose [oral glucose tolerance test (OGTT)]. On 1 of the study days (OGTT+F), the subject received 7.5 g fructose (Sigma, St. Louis, MO) in addition to the 75 g glucose, and on the other day the subject received no fructose (OGTT-F). Blood samples were drawn every 15 min for 120 min after ingestion of the carbohydrate.

Analytical methods

Plasma glucose concentrations were measured with the glucose oxidase technique using a Glucose Analyzer II (Beckman Coulter, Inc., Fullerton, CA). Plasma insulin and glucagon (using 30-K antiserum) were measured by RIA (7, 8). Lactate (9) and fructose (10) were measured in blood deproteinized with perchloric acid. Plasma nonesterified fatty acids (NEFA) and triglycerides were measured with enzymatic colorimetric assays (NEFA C, Wako Chemicals, Richmond, VA, and IL Test Triglyceride, Instrumentation Laboratory, Lexington, MA, respectively) on a Monarch 2000 centrifugal analyzer (Instrumentation Laboratory).

Calculations and statistical analysis

Data are the mean ± SE. The trapezoidal rule was used for calculation of areas under the curve (AUC) of substrate and hormone responses. All AUC are incremental (*i.e.* change from baseline values). Paired Student's *t* tests were used for analysis of AUC data. Time-course data were analyzed with repeated measures ANOVA. Data were accepted as significant at $P < 0.05$.

Results

Glucose response

There were no significant differences in the peak plasma glucose concentrations during the OGTT-F and the OGTT+F (Fig. 1A). The AUC of the plasma glucose response, calculated as the change from basal values in each subject, was approximately 19% smaller during the OGTT+F than during the OGTT-F ($P < 0.05$; Fig. 1B).

Glucose tolerance was improved by fructose in nine subjects and was worsened by fructose in two subjects. Subjects with the largest glucose AUC in response to the OGTT-F demonstrated the greatest improvement in glucose tolerance with the administration of fructose, *i.e.* there was a positive correlation between the AUC of the glycemic response to the OGTT-F and the difference between the OGTT-F AUC and the OGTT+F AUC (r = 0.72; $P < 0.05$; Fig. 2). There was no significant effect of order of study on the glycemic response ($P < 0.3$).

Insulin and glucagon

The insulin concentrations did not differ significantly between the OGTT-F and OGTT+F (AUC, 24,732 ± 4,800 and 27,372 ± 4,572 pmol/L, respectively; Fig. 3A). Of the nine subjects with a smaller glucose AUC during the OGTT+F than during the OGTT-F, five had a smaller insulin AUC during the OGTT+F (OGTT+F minus OGTT-F = −3,372 ± 1,536 pmol/L), and four had a larger insulin AUC during the OGTT+F (OGTT+F minus OGTT-F = 4,392 ± 1,686

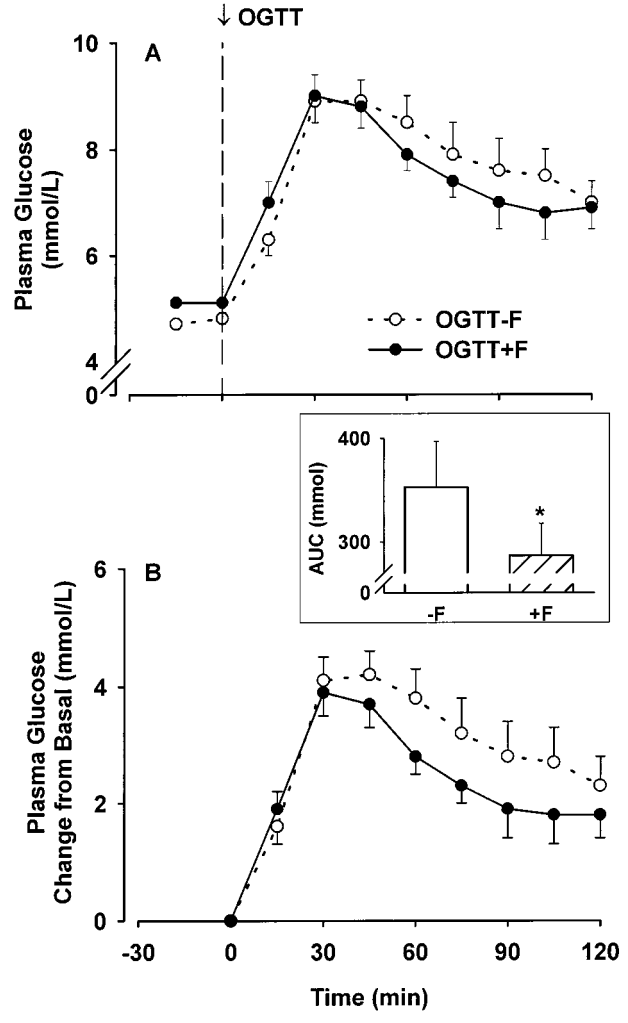

FIG. 1. Plasma glucose concentrations (A) and change from basal in plasma glucose concentrations (B) in 11 healthy adults during a 75-g oral glucose tolerance test without (OGTT-F) or with (OGTT+F) the addition of 7.5 g fructose to the glucose load. The *inset* shows the AUC of the change from basal glucose concentrations. *, $P < 0.05$ between OGTTs.

pmol/L). Two subjects had greater insulin and glucose responses during the OGTT+F than during the OGTT-F. Both of these subjects exhibited an increase of about 30% in the AUC of the glucose response, with a 50–100% increase in the AUC of the insulin response.

Basal glucagon concentrations were 47 ± 3 and 50 ± 5 ng/L in the OGTT-F and OGTT+F, respectively (Fig. 3B), and the concentrations declined similarly in both studies (final concentrations, 40 ± 3 and 41 ± 5 ng/L, respectively; P = NS *vs.* basal or between studies).

Fructose and lactate

The blood fructose concentrations were nearly twice as great during the study with fructose as in the one without fructose (20.7 ± 3.9 and 38.4 ± 3.8 μmol/L in OGTT-F and OGTT+F, respectively; $P < 0.05$; Fig. 4A).

The AUC of the change in blood lactate after carbohydrate ingestion was approximately 50% greater in OGTT-F than in OGTT+F (44.3 ± 11.4 and 65.8 ± 7.5 mmol/L in OGTT-F and OGTT+F, respectively; $P < 0.05$; Fig. 4B).

NEFA and triglycerides

Neither NEFA nor triglyceride concentrations differed at any time between the two studies (Table 1). During the postprandial period, NEFA concentrations declined in relation to basal values during both OGTTs. Triglyceride concentrations declined significantly from baseline only during the OGTT-F.

Discussion

The subjects in the current study received a small catalytic amount of fructose (7.5 g, approximately the amount in 100 g grapes or apple) (11). Addition of fructose to the glucose load significantly reduced the glycemic response to the OGTT, a finding that was especially impressive because the subjects received 10% more carbohydrate during the OGTT+F than during the OGTT-F. The increase in the carbohydrate load

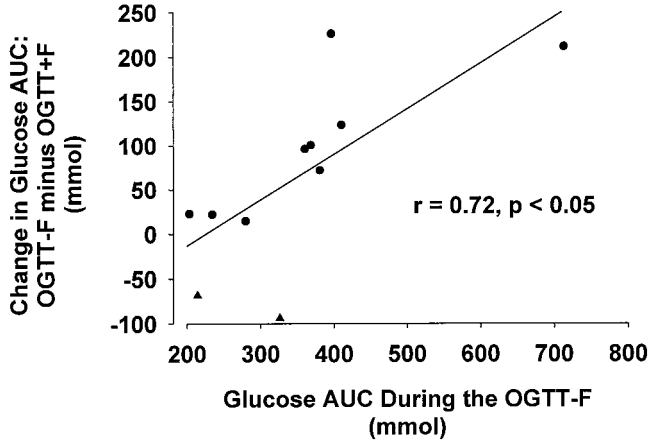

FIG. 2. A significant positive relationship existed between the magnitude of the glycemic response during a 75-g oral glucose tolerance test (OGTT-F; plotted on the *x*-axis) and the improvement in glucose tolerance during the OGTT+F (*i.e.* the glucose AUC during the OGTT-F minus that during the OGTT+F; plotted on the *y*-axis). The two subjects shown with *triangles* are those who failed to exhibit a decrease in the AUC of the glucose response during the OGTT+F *vs.* the OGTT-F.

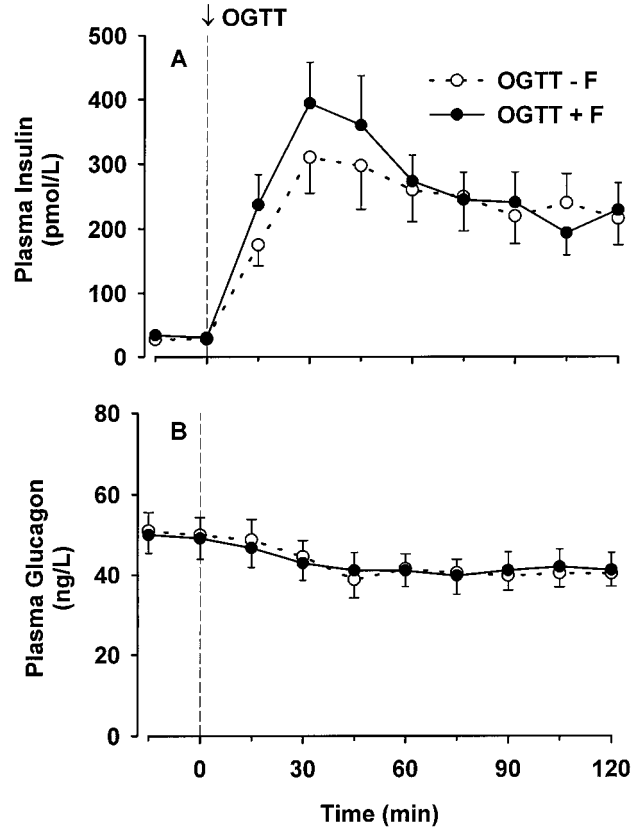

FIG. 3. Plasma insulin (A) and glucagon (B) during a 75-g oral glucose tolerance test without (OGTT-F) or with (OGTT+F) the addition of 7.5 g fructose to the glucose load. There were no significant differences in the AUC of the response over the 120-min period (using paired *t* test) or at any time point (using repeated measures ANOVA) between treatments.

during the OGTT+F was by design, because the goal was to determine the effect of fructose *per se* on glucose tolerance, rather than the effect of fructose as a replacement for other dietary carbohydrates.

This study was not designed to examine the mechanism(s) by which fructose had its effect, but differences in the insulin response during the two OGTT and the stimulation of hepatic glucose uptake secondary to enhanced hepatic glucokinase translocation are two possibilities. If the 120-min postingestion period is considered as a whole, there were no significant differences in the insulin responses between OGTTs. However, there was a tendency for the insulin concentrations to be higher in the OGTT+F *vs.* the OGTT-F for the first 45 min after carbohydrate ingestion. If a paired *t* test were used to compare the insulin concentrations at the 30 min point, the concentrations would have been significantly higher during the OGTT+F *vs.* OGTT-F. Similarly, the AUC of the insulin response during the period between 0 and 45 min was about 25% greater during the OGTT+F than during the OGTT-F ($P < 0.05$). Higher insulin concentrations would have stimulated glucose uptake by both the liver and insulin-responsive peripheral tissues, reducing the glycemic response. Nevertheless, there were no significant correlations between the insulin responses and the glycemic responses (whether the 0–45 min or the 0–120 min time period is considered). Of the nine subjects who demonstrated an improvement in glucose tolerance with fructose administration,

five had a smaller insulin response, and four had a larger insulin response during the OGTT+F than during the OGTT-F. The two subjects who exhibited poorer glucose tolerance during the OGTT+F than during the OGTT-F had insulin responses that were 22–48% larger during the OGTT+F than during the OGTT-F. Thus, there was little evidence that stimulation of insulin secretion was responsible for the smaller glycemic response in most subjects during the OGTT+F.

In regard to the second possible mechanism for the fructose effect (stimulation of glucokinase translocation), it is known that the liver is a major contributor to the disposition of enterally delivered glucose, taking up 20–30% of absorbed glucose in healthy humans and converting approximately 70% of the glucose to glycogen (12). Phosphorylation of glucose by glucokinase is a rate-determining step for hepatic glucose metabolism. In the basal (unfed) state, glucokinase in the liver is localized primarily in the nucleus, where it is bound to the glucokinase regulatory protein (GKRP). When GKRP is bound to fructose-6-phosphate, it is in a conformation that favors interaction with glucokinase. On the other hand, fructose-1-phosphate competes with fructose-6-phosphate for binding to GKRP, and in so doing, releases glucokinase from GKRP (13). Intraportal fructose infusion for 270 min increased the hepatic concentration of fructose-1-phosphate to more than 170% of the basal level in conscious dogs (10). Fructose administration in animal models stimulates the translocation of glucokinase (Shiota, M., P. Galassetti, T. L. Jetton, M. A. Magnuson, and A. D. Cherrington, unpublished observations), and low dose fructose administration accompanying intraduodenal glucose infusion in dogs enhances net hepatic glucose uptake and net hepatic fractional extraction of glucose about 2-fold (5). The difference between the AUC of the plasma glucose responses during the OGTT+F and OGTT-F totaled approximately 66 mmol. Blood lactate concentrations were higher after the OGTT+F than the OGTT-F ($P < 0.05$), presumably because of an increase in net hepatic production of lactate (5). The difference in the lactate AUC between the two tests totaled about 11 mmol glucose equivalents, consistent with a stimulation of glycolysis. Fructose is reported to increase the activities of both pyruvate kinase and phosphofructokinase, key regulators of glycolytic flux (14–17). Thus, the difference in the glucose AUC between the OGTTs was much greater than the difference in the lactate AUC (~55 mmol). Although the fate of this carbon is unclear, the findings are consistent with enhancement of NHGU and glycogen storage by fructose. In the presence of low dose fructose infusion, humans subjected to a euglycemic hyperinsulinemic clamp exhibit enhanced glycogen synthase flux and hepatic glycogen storage (18).

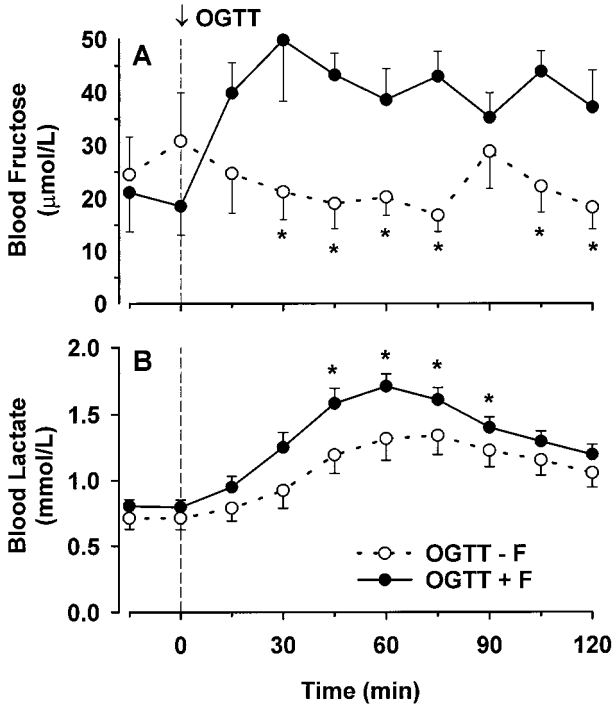

FIG. 4. Blood fructose (A) and lactate (B) concentrations during a 75-g oral glucose tolerance test without (OGTT-F) or with (OGTT+F) the addition of 7.5 g fructose to the glucose load. *, Significantly higher during the OGTT+F than the OGTT-F ($P < 0.05$).

The improvement in glucose tolerance with fructose administration was greatest in those individuals with the largest glycemic excursion in response to the OGTT-F. The subjects could be divided into those with large responses to the OGTT-F (defined as >330 mmol/L) and those with small responses to the OGTT-F (<330 mmol/L). All 6 subjects with a glucose AUC greater than 330 mmol/L during the OGTT-F exhibited a smaller glycemic response during the OGTT+F. On the other hand, only 3 of the 5 subjects with a glucose AUC less than 330 mmol/L during the OGTT-F had a smaller glycemic response during the OGTT+F, and the reduction in the glycemic response in those 3 subjects was modest. The difference in the glucose responses between the 2 subgroups can be seen more clearly in Fig. 5, which depicts the time course of the glucose response. None of the 11 subjects had impaired glucose toler-

TABLE 1. Plasma NEFA and triglyceride concentrations during a 75-g oral glucose tolerance test without (OGTT-F) or with (OGTT + F) the addition of 7.5 g fructose to the glucose load

Parameter and treatment	Basal	Min after ingestion of carbohydrate		
		30	60	120
NEFA (μmol/L)				
OGTT − F	443 ± 54	290 ± 36[a]	136 ± 21[a]	78 ± 6[a]
OGTT + F	381 ± 55	230 ± 28[a]	112 ± 11[a]	84 ± 7[a]
Triglycerides (g/L)				
OGTT − F	1.01 ± 0.15	1.01 ± 0.13	0.98 ± 0.13	0.85 ± 0.13[a]
OGTT + F	0.96 ± 0.07	0.88 ± 0.07	0.99 ± 0.09	0.94 ± 0.08

Values are the mean ± SEM. The 11 subjects received both OGTT, in random order. There are no significant differences between OGTT − F and OGTT + F.

[a] $P < 0.05$ *vs.* basal values during the same OGTT.

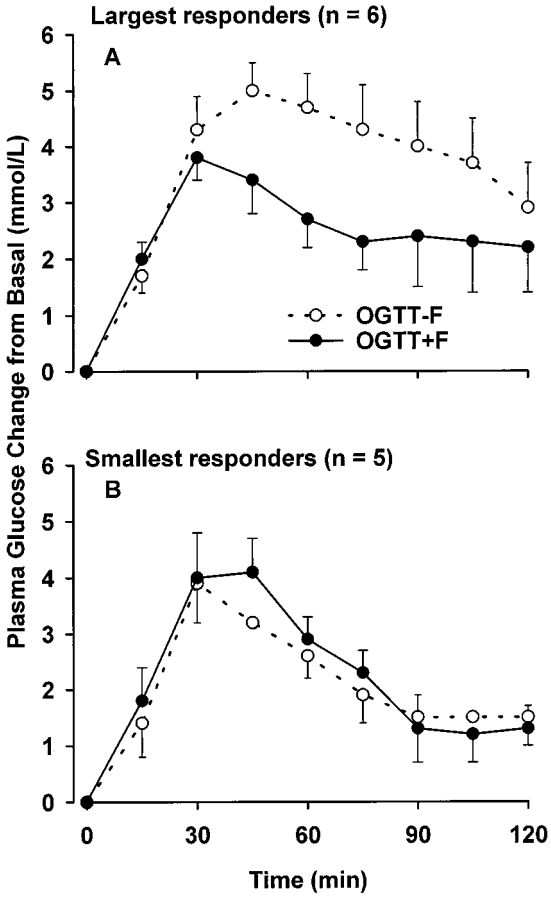

FIG. 5. The group could be divided into two subgroups, based on their glucose AUC during the OGTT-F. The six individuals with the largest response to the OGTT-F (>330 mmol; shown in A) showed a significant improvement in glucose tolerance during the OGTT+F. The five subjects with a small glucose AUC (<330 mmol) in response to the OGTT-F (B) did not improve with fructose administration.

ance or frank diabetes, as defined by the American Diabetes Association criteria (19). Even in these individuals with normal glucose tolerance, however, those with the poorest glucose tolerance benefited the most from the addition of fructose to the glucose load. These results suggest that individuals with abnormal glucose tolerance might benefit even more than subjects with normal glucose tolerance from the addition of fructose to glucose feedings. The 2 subjects who exhibited an increase in the AUC of the glucose response during the OGTT+F *vs.* the OGTT-F were both Asian males, but we know of no data indicating that there are racial or gender differences in fructose metabolism. These subjects were similar in age (22 and 31 yr) and body mass index (22.2 and 23.3 kg/m^2) to the balance of the subjects. The third Asian subject, a woman, demonstrated a 57% decrease in the glucose AUC during the OGTT+F compared with that during the OGTT-F.

During both OGTTs the hyperglycemic, hyperinsulinemic conditions suppressed lipolysis significantly and to a similar extent, based on NEFA concentrations. Both chronic and acute consumptions of large amounts of fructose (~20% of total energy intake and 0.75 g/kg BW, respectively) are reported to elevate circulating triglyceride levels (20, 21). In response to a low dose of fructose, triglyceride concentrations remained at basal levels. On the other hand, triglyceride concentrations declined significantly during the OGTT-F, suggesting that there was a mild stimulation of triglyceride synthesis in response to fructose (22).

In conclusion, the addition of small (catalytic) amounts of fructose to a glucose load improves glucose tolerance in normal humans, with the improvement being most evident in those individuals with the worst (albeit not clinically abnormal) glucose tolerance. The improvement in glucose tolerance cannot be explained on the basis of the insulin response to the carbohydrate loads. These findings may hold promise for the improvement of carbohydrate tolerance in individuals with impaired glucose tolerance and diabetes.

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References

1. **Crapo PA, Kolterman OG, Olefsky JM.** 1980 Effects of oral fructose in normal, diabetic, and impaired glucose tolerance subjects. Diabetes Care. 3:575–582.
2. **Henry RR, Crapo PA.** 1991 Current issues in fructose metabolism. Annu Rev Nutr. 11:21–39.
3. **Uusitupa MIJ.** 1994 Fructose in the diabetic diet. Am J Clin Nutr. 59:753S–757S.
4. **Thorburn AW, Crapo PA, Griver K, Wallace P, Henry RR.** 1989 Long-term effect of dietary fructose on carbohydrate metabolism in non-insulin dependent diabetes mellitus. Am J Clin Nutr. 49:1155–1163.
5. **Shiota M, Galassetti P, Moore MC, Monohan M, Cherrington AD.** 1997 Small amounts of fructose markedly improve glucose tolerance in the dog. Diabetes. 46:49.A.
6. **Abumrad NN, Rabin D, Diamond M, Lacy W.** 1981 Use of a heated superficial hand vein as an alternative site for measurement of amino acid concentrations and for the study of glucose and alanine kinetics in man. Metabolism. 30:936–940.
7. **Morgan CR, Lazarow AL.** 1963 Immunoassay of insulin: two antibody system. Plasma insulin of normal, subdiabetic and diabetic rats. Diabetes. 12:115–126.
8. **Aguilar-Parada E, Eisentraut EM, Unger RH.** 1969 Pancreatic glucagon secretion in normal and diabetic subjects. Am J Clin Sci. 257:415–419.
9. **Lloyd B, Burrin J, Smythe P, Alberti KGMM.** 1978 Enzymatic fluorometric continuous-flow assays of blood glucose, lactate, pyruvate, alanine, glycerol, and 3-hydroxybutyrate. Clin Chem. 24:1724–1729.
10. **Shiota M, Galassetti P, Monohan M, Neal DW, Cherrington AD.** 1998 Small amounts of fructose markedly augment net hepatic glucose uptake in the conscious dog. Diabetes. 47:867–873.
11. **Holland B, Unwin ID, Buss DH.** 1992 Fruits and nuts. In: McCance and Widdowson's the composition of foods, 5th Ed, Suppl 1. Cambridge: Royal Society of Chemistry and Ministry of Agriculture, Fisheries and Food.
12. **Pagliassotti MJ, Horton TJ.** 1994 Hormonal and neural regulation of hepatic glucose uptake. In: Pagliassotti MJ, Davis SN, Cherrington AD, eds. The role of the liver in maintaining glucose homeostasis. Austin: Landes; 45–84.
13. **Van Schaftingen E, Detheux M, Veiga Da Cunha M.** 1994 Short-term control of glucokinase activity: role of a regulatory protein. FASEB J. 9:414–419.
14. **Eggleston LV, Woods HF.** 1970 Activation of liver pyruvate kinase by fructose-1-phosphate. FEBS Lett. 6:43–45.
15. **Fillat C, Gomez-Foix AM, Guinovart JJ.** 1993 Stimulation of glucose utilization by fructose in isolated rat hepatocytes. Arch Biochem Biophys. 300:564–569.
16. **Hue L, Rider MH.** 1987 Role of fructose 2,6-bisphosphate in the control of glycolysis in mammalian tissues. Biochem J. 245:313–324.
17. **Ogawa A, Nishi T, Furuya E, et al.** 1993 The effect of fructose on fructose 2,6-bisphosphate level and fructose 6-phosphate, 2-kinase activity in the perfused rat liver. Biochem Mol Biol Int. 30:83–90.
18. **Petersen KMF, Laurent D, Cline GW.** 2000 Effect of low dose fructose on hepatic glycogen synthase and phosphorylase flux in man assessed by ^{13}C NMR. Diabetes. 49:A301.
19. **Expert Committee on the Diagnosis and Classification of Diabetes Mellitus.** 2000. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care. 23:S4–S19.
20. **Bantle JP, Swanson JE, Thomas W, Laine DC.** 1992 Metabolic effects of dietary fructose in diabetic subjects. Diabetes Care. 15:1468–1476.
21. **Abraha A, Humphreys SM, Clark ML, Matthews DR, Frayn KN.** 1998 Acute effect of fructose on postprandial lipaemia in diabetic and non-diabetic subjects. Br J Nutr. 80:169–175.
22. **Hellerstein MK.** 1999 De novo lipogenesis in humans: metabolic and regulatory aspects. Eur J Clin Nutr. 53(Suppl 1):S53–S65.