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Comparison of the effects of sucrose and fructose on insulin action and glucose tolerance

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Thresher, Jeffrey S., Deborah A. Podolin, Yuren Wei, Robert S. Mazzeo, and Michael J. Pagliassotti. Comparison of the effects of sucrose and fructose on insulin action and glucose tolerance. *Am J Physiol Regulatory Integrative Comp Physiol* 279: R1334–R1340, 2000.—The purpose of the present study was to determine whether fructose is the nutrient mediator of sucrose-induced insulin resistance and glucose intolerance. Toward this end, male rats were fed a purified starch diet (68% of total calories) for a 2-wk baseline period. After this, rats either remained on the starch (ST) diet or were switched to a sucrose (SU, 68% of total calories), fructose/glucose (F/G, 34/34% of total calories), or fructose/starch (F/ST, 34/34% of total calories) diet for 5 wk. Rats then underwent either an intravenous glucose tolerance test (n = 10/diet) or a euglycemic, hyperinsulinemic clamp (n = 8 or 9/diet). Incremental glucose and insulin areas under the curve in SU, F/G, and F/ST were on average 61 and 29% greater than ST, respectively, but not significantly different from one another. During clamps, glucose infusion rates ($mg \cdot kg^{-1} \cdot min^{-1}$) required to maintain euglycemia were significantly lower ($P < 0.05$) in SU, F/G, and F/ST (13.4 ± 0.9, 9.5 ± 1.7, 11.3 ± 1.3, respectively) compared with ST (22.8 ± 1.1). Insulin suppression of glucose appearance ($mg \cdot kg^{-1} \cdot min^{-1}$) was significantly lower ($P < 0.05$) in SU, F/G, and F/ST (5.6 ± 0.5, 2.2 ± 1.2, and 6.6 ± 0.7, respectively) compared with ST (9.6 ± 0.4). Insulin-stimulated glucose disappearance ($mg \cdot kg^{-1} \cdot min^{-1}$) was significantly lower ($P < 0.05$) in SU, F/G, and F/ST (17.9 ± 0.6, 16.2 ± 1.3, 15.3 ± 1.8, respectively) compared with ST (24.7 ± 1.2). These data suggest that fructose is the primary nutrient mediator of sucrose-induced insulin resistance and glucose intolerance.

diet composition

INSULIN RESISTANCE (reduced insulin suppression of hepatic glucose production and reduced insulin stimulation of glucose uptake) is a characteristic feature of type 2 diabetes. The development of insulin resistance can be linked to both genetic and environmental factors (8). A key environmental factor in the development of insulin resistance and the focus of this study is diet composition. Diet-induced insulin resistance is characterized by fasting normoglycemia accompanied by hyperinsulinemia, impaired insulin suppression of hepatic glucose production, and impaired insulin-stimulated glucose uptake in muscle and adipose tissue (13, 19, 20).

High-sucrose diets have been shown to impair insulin action in rats at both the hepatic and peripheral levels (13, 16, 19). During absorption, sucrose is hydrolyzed into equal quantities of fructose and glucose. Consequently, high-fructose diets have been directly compared with high-glucose or -starch diets in an attempt to distinguish which moiety of the sucrose disaccharide may be a factor in sucrose-induced insulin resistance in rats (20, 21). For example, Thorburn et al. (20) showed that a diet containing 35% fructose produced insulin resistance, whereas a diet containing 35% glucose did not. Studies such as these have led to the conclusion that fructose is responsible for sucrose-induced insulin resistance.

However, there is reason to believe that the degree of insulin resistance produced by fructose may be less than that from sucrose. Storlien et al. (19) fed male rats either a high-sucrose (69% sucrose) or high-starch (69% starch) diet for 4 wk. This study demonstrated that impaired insulin suppression of glucose production in the sucrose-fed animals was responsible for 64% of the difference seen in the glucose infusion rate between the sucrose- and starch-fed animals. In contrast, the aforementioned study by Thorburn et al. (20) demonstrated that impaired insulin suppression of glucose production in the fructose-fed animals was responsible for only 44% of the difference seen in the glucose infusion rate between the fructose- and starch-fed animals. These data suggest that the relative contributions of hepatic and peripheral insulin resistance may be different between sucrose- and fructose-containing diets. These discrepancies in no way detract from the validity of these studies but do illustrate the need for a single study that compares the effects of sucrose, fructose and glucose, and fructose and starch on hepatic and peripheral insulin action.

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Impaired glucose tolerance is a risk factor for the development of type 2 diabetes. Furthermore, impaired glucose tolerance has been observed in rats fed a high-sucrose diet for 90–120 days (11). It is not clear from the literature whether the impaired glucose tolerance results from the metabolism of fructose alone or the combined effects of both fructose and glucose.

Therefore, the specific aims of the present study were *1*) to compare the effects of dietary sucrose, fructose and glucose, and fructose and starch on whole body insulin action and *2*) to study the effects of diet-induced insulin resistance on glucose tolerance. To achieve these aims, the euglycemic-hyperinsulinemic clamp technique was used to assess whole body insulin action and an intravenous glucose tolerance test was used to assess glucose tolerance.

MATERIALS AND METHODS

Animals and Housing

Male Crl:(WI)BR rats (Sasco, Madison, WI) weighing 140–160 g were used in all experiments. The animals were individually housed in a thermally controlled environment with a 12:12-h light-dark cycle. These conditions, as well as the treatment of the animals, met guidelines set by the American Association of Laboratory Animal Care and all protocols were approved by the University of Colorado Health Sciences Center Animal Care Committee.

Diet Protocol

All animals were fed ad libitum a purified high-starch diet (ST: 68% corn starch, 12% fat, 20% protein; Table 1, Research Diets, New Brunswick, NJ) for a baseline period of 2 wk. After the baseline period, the rats were randomly assigned to either a control group (ST) or one of three dietary groups. The control group continued to be fed the ST diet for an additional 5-wk study period. The dietary groups were fed either a high-sucrose (SU: 68% sucrose, 12% fat, 20% protein), a 34% fructose, 34% glucose, 12% fat, and 20% protein (F/G), or 34% fructose, 34% starch, 12% fat, and 20% protein (F/ST) diet (Table 1) for an additional 5-wk study period. The F/G diet was chosen to test if both products of sucrose absorption (i.e., fructose and glucose) are required to produce an insulin-resistant state comparable to that of sucrose. The F/ST diet was chosen to test whether fructose alone would result in an insulin-resistant state similar to that caused by the SU diet. Starch was used instead of glucose in F/ST diet because starch is absorbed more slowly than glucose (7). Moreover, when starch is provided as the sole carbohydrate source, insulin action on glucose metabolism is not impaired (14, 16). Food intake was measured three times per week and body weight was measured once a week throughout the baseline and 5-wk dietary period.

Table 1. *Diet composition*

	ST	SU	F/G	F/ST
Casein	200	200	200	200
DL-methionine	3	3	3	3
Cornstarch	500	0	0	175
Maltodextrin 10	150	0	0	150
Sucrose	0	650	0	0
Fructose	0	0	325	325
Glucose	0	0	325	0
Cellulose	50	50	50	50
Corn oil	50	50	50	50
Salt mix	35	35	35	35
Vitamin mix	10	10	10	10
Choline bitartrate	2	2	2	2

Values are in g/kg. ST, starch; SU, sucrose; F/G, fructose/glucose. Maltodextrin 10 is enzyme converted cornstarch with a dextrose equivalence of 10% (all diets were fabricated by Research Diets, New Brunswick, NJ).

Surgical Procedures

After the 5-wk dietary period, rats from each group were randomly assigned to undergo either an intravenous glucose tolerance test (IVGTT, $n = 40$) or a euglycemic, hyperinsulinemic clamp ($n = 35$). In preparation for experiments, the animals were anesthetized (intramuscular injection of acepromazine, 5 mg/kg; xylazine, 10 mg/kg; ketamine, 50 mg/kg) and cannulas (polyethylene tubing, 0.022 in. ID, PE-50 Intramedic Clay Adams, Parsippany, NJ) were inserted into the left carotid artery and advanced to the aortic arch and into the right jugular vein and advanced to the vena cava (17). Cannulas were then sutured to their respective vessels and exteriorized out the back of the neck of the animal. The cannulas were flushed and then filled with heparinized saline and sealed. The rats were allowed 4 days to recover and were at >90% of their presurgery body weight on the day of the study. This results in animals that are healthy based on the following parameters: energy intake, normal stools, and white blood cell count. During recovery, the animals were fed their respective diets ad libitum.

Study 1: IVGTT

The IVGTT was used to assess glucose tolerance and the glucose and insulin response to a glucose injection. Animals were fasted for a 24-h period to ensure that no food was being absorbed and that liver glycogen levels were at a stable minimum (13). On the day of study, extensions were added to the catheters to allow easy access without disturbing the animal. Animals were then allowed to rest for 30 min. A baseline blood sample was then taken. A glucose bolus of 0.4 g/kg body wt was injected into the venous catheter over the course of 1 min. Blood samples were taken at the following times after administration of the bolus: 5, 12.5, 20, 27.5, 35, 50, 65, and 80 min. Blood sample volumes were 250 μl, except for the 0, 20, 50, and 80 min times where they were 400 μl. This sampling protocol resulted in the removal of ≈8.9% of the animal's blood volume (assumes blood volume = 8% of body wt). Blood samples were immediately centrifuged, and plasma was analyzed for glucose on the day of the study. Plasma for insulin (all times) and triglyceride (0 min time point) analyses was frozen at −70°C. After 80 min, the animal was anesthetized with pentobarbital sodium (70 mg/kg iv). Retroperitoneal, mesenteric, and epididymal fat pads were removed and weighed. The sum of these fat pads correlates with total carcass lipid ($r = 0.93$, $P < 0.001$) (M. J. Pagliassotti and E. C. Gayles, unpublished data). In addition, urine samples were taken and immediately analyzed for glucose to monitor renal spillage of glucose during the IVGTT.

Study 2: Euglycemic, Hyperinsulinemic Clamps

The euglycemic, hyperinsulinemic clamp procedure was used to determine the magnitude and tissue distribution of insulin action. The clamp protocol included both a basal (no exogenous insulin) and hyperinsulinemic (4× basal insulin

infusion) period that enabled each rat to serve as its own control. Rats were fasted for 6–8 h to ensure that animals were in a postabsorptive state but with significant amounts of liver glycogen still available (13). As in the IVGTT, extensions were added to the catheters and the animal was allowed to rest for 30 min. A baseline blood sample (300 μl) was taken, and a primed, continuous infusion of HPLC-purified [3-^{3}H]glucose (prime of 12 μCi, infusion of 0.1 μCi/min) was initiated. Arterial blood samples (300 μl) were taken at 45, 52.5, and 60 min. After the final basal sample, an insulin infusion was initiated (4 $mU \cdot kg^{-1} \cdot min^{-1}$) to elicit an approximate fourfold increase in the systemic insulin concentration (15). A variable exogenous glucose infusion (10% dextrose) was then started to clamp glucose at basal levels. The glucose infusate was spiked with [3-^{3}H]glucose to give a specific activity in the infusate that was 50% of that expected in the plasma during the final 15 min of the basal period (45–60 min). The specific activity of the glucose infusate was chosen to account for the reduction in glucose rate of appearance due to hyperinsulinemia (1). This procedure minimized changes in glucose specific activity and allowed for accurate estimates of glucose kinetics (6). During the clamp, arterial blood samples (25 μl) were taken at 5-min intervals for immediate glucose analysis. Manual adjustments to the glucose infusion rate were then made to maintain euglycemia throughout the clamp. During the final 15-min steady-state period, larger arterial samples (300 μl) were taken (120, 127.5, and 135 min). This sampling protocol resulted in the removal of ≈12% of the animal's blood volume. All blood samples were immediately centrifuged, and the plasma was used to determine glucose concentration. Plasma from the basal steady-state period and the clamp steady-state period were used to determine glucose radioactivity and insulin concentration. After the final sample, animals were anesthetized (70 mg/kg iv pentobarbital sodium) and the entire gastrocnemius, soleus, and vastus lateralis muscles from the right hindquarter and a portion of the abdominal wall muscle were removed and immediately frozen in liquid nitrogen. These tissues were analyzed for glycogen and tracer content. Epididymal, retroperitoneal, and mesenteric fat pads were removed and weighed.

Analytic Methods

Plasma samples for tracer analysis were deproteinized overnight with $ZnSO_4$ and $Ba(OH)_2$. The samples were then centrifuged, dried to eliminate tritiated water, reconstituted with distilled H_20, and counted in a liquid scintillation counter (Beckman Instruments, Fullerton, CA). A Beckman glucose analyzer was used to determine the plasma glucose concentration. Plasma triglycerides were measured with Sigma kit 320-A (St. Louis, MO). Plasma insulin was determined by radioimmunoassay (Linco Research, St. Charles, MO). Muscle glycogen concentration and glycogen radioactivity were measured on homogenates from the entire amount of tissue removed at the time of death according to the procedures of Chan and Exton (2). Tissue triglycerides were determined after extraction (4), and hydrolysis was determined by enzymatic assay as described above (Sigma).

Calculations

IVGTT. Insulin and glucose areas under the curve (AUC) were measured using the trapezoid rule. Only positive excursions from the baseline were included in the calculations.

Euglycemic, hyperinsulinemic clamps. During the baseline period, rates of glucose appearance (R_a) and disappearance (R_d) were estimated by isotope dilution using a constant infusion of HPLC-purified D-[3-^{3}H]glucose (3). Steady-state equations were used because the glucose specific activity changed <0.1%/min during the sampling period. During the clamp, R_a and R_d were estimated based on the procedures of Finegood and Bergman (5).

Glycogen synthetic rate. Glycogen synthetic rate was calculated as the ratio of the average amount of [^{3}H]glycogen (dpm/g tissue) in soleus, gastrocnemius, vastus lateralis, and abdominal muscles to the steady-state glucose specific activity. This value was divided by the length of the clamp period in minutes and multiplied by 1,000 to convert grams to kilograms. These data were used as a measure of insulin-stimulated glycogen synthesis in muscle tissue. Previous data demonstrated that negligible incorporation of [3-^{3}H]glucose into muscle glycogen occurs under basal conditions (15).

Statistics

Analysis of variance was used to compare the following endpoints among groups: body weight, energy intake, fat pad weight, circulating metabolites (except glucose, see below) and hormones, AUC, and clamp data. Statistical significance was accepted at $P < 0.05$. Where appropriate, the Student-Newman-Keuls test was used for post hoc analysis. SE was calculated using an approximation when groups were of unequal size (22). Plasma glucose data was characterized by unequal variances. To compare differences among groups for this parameter, the Kruskal-Wallis statistic was used. Data are presented as means ± SE.

RESULTS

Study 1: IVGTT

Body weight and energy intake. Body weight (pooled mean = 213.1 ± 1.3 g) at the start of the 5-wk dietary period and energy intake (pooled mean = 2,835.7 ± 36.7 kcal) during the 5-wk period were not significantly different among groups. Weight gain during the 5-wk dietary period was significantly less in F/G (164.0 ± 10.0 g) compared with ST (199.2 ± 11.6 g) and F/ST (197.6 ± 9.6 g) but was not significantly different from SU (177.0 ± 4.8 g). There were no significant differences in fat pad mass among groups (data not shown).

Fasting glucose, insulin, and triglycerides. Fasting plasma glucose levels were not significantly different among groups. Fasting plasma insulin and triglyceride levels were significantly higher in SU, F/G, and F/ST compared with ST. Fasting plasma triglycerides were significantly higher in F/ST compared with SU and F/G (Table 2).

Table 2. *Fasting plasma glucose, insulin, and triglyceride levels before IVGTT*

Diet	Plasma Glucose, mg/dl	Plasma Insulin, μU/ml	Plasma Triglycerides, mg/dl
ST	116.9 ± 3.0	23.6 ± 1.9	72.8 ± 4.6
SU	118.8 ± 3.5	46.2 ± 2.2*	132.8 ± 7.1*†
F/G	113.9 ± 1.3	48.2 ± 2.8*	127.8 ± 7.5*†
F/ST	121.2 ± 4.5	49.6 ± 2.3*	158.5 ± 5.6*

Values are means ± SE for 10 rats per group. IVGTT, intravenous glucose tolerance test. *Significantly different from ST ($P < 0.05$). †Significantly different from F/ST ($P < 0.05$).

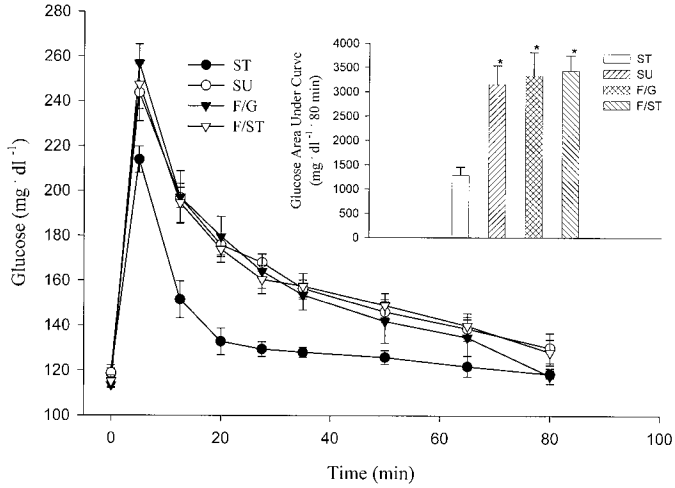

Fig. 1. Plot of plasma glucose (mg/dl) across time for starch (ST)-, sucrose (SU)-, fructose/glucose (F/G)-, and F/ST-fed rats in response to a glucose bolus of 0.4 g/kg body wt injected intravenously. Values are means ± SE for 10 rats/group. *Inset*: glucose incremental area under the curve. Values are means ± SE for 10 rats/group. *Significantly different from ST-fed animals ($P < 0.05$).

Glucose and insulin response to IVGTT. The incremental area under the glucose curve (GAUC) was significantly greater in SU, F/G, F/ST compared with ST. The incremental area under the insulin curve (IAUC) was significantly greater in SU compared with ST. IAUC was not significantly different among ST, F/G, and F/ST. A total of 21 rats (ST, $n = 1$; SU, $n = 6$; F/G, $n = 7$; F/ST, $n = 7$) did not return to baseline glucose levels after the 80-min IVGTT period. There were no significant differences in urinary glucose among groups and urinary glucose represented ≤0.01% of the glucose bolus given during the IVGTT (Figs. 1 and 2).

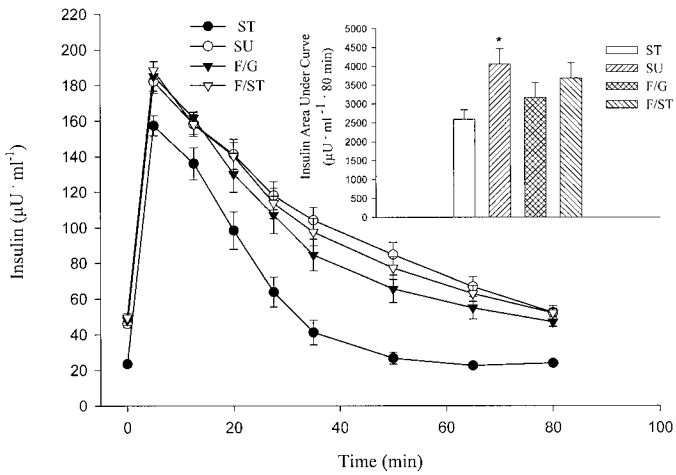

Fig. 2. Plot of plasma insulin (μU/ml) across time for ST-, SU-, F/G-, and F/ST-fed rats in response to a glucose bolus of 0.4 g/kg body wt injected intravenously. Values are means ± SE for 10 rats/group. *Inset*: insulin incremental area under the curve. Values are means ± SE for 10 rats/group. *Significantly different from ST-fed animals ($P < 0.05$).

Table 3. *Clamp fat pad weight*

Diet	Epididymal Fat Pad Wt	Retroperitoneal Fat Pad Wt	Mesenteric Fat Pad Wt	Fat Sum
ST	6.7 ± 1.1	5.5 ± 0.9	3.1 ± 0.6	15.2 ± 2.6
SU	5.7 ± 0.6	5.2 ± 0.8	2.6 ± 0.4	13.5 ± 1.6
F/G	7.8 ± 0.5	6.0 ± 0.4	3.6 ± 0.3	17.3 ± 0.9
F/ST	10.0 ± 0.8*	7.3 ± 0.9	3.9 ± 0.4	21.2 ± 1.9*

Values are mean ± SE in g ($n = 9$ ST, SU, F/G; $n = 8$ F/ST). Fat sum is sum of epididymal, retroperitoneal, and mesenteric fat pads. *Significantly different from SU ($P < 0.05$).

Study 2: Hyperinsulinemic, Euglycemic Clamps

Body weight and energy intake. Body weight (pooled mean = 230.1 ± 0.4 g) at the start of the 5-wk dietary protocol and weight gain (pooled mean = 191.7 ± 0.7 g) during the 5-wk period were not significantly different among groups. Total energy intake during the 5-wk dietary period in F/G (2,740.5 ± 92.4 kcal) and F/ST (2,846.4 ± 128.9 kcal) was significantly higher compared with SU (2,409.1 ± 69.5 kcal). The weight of the epididymal fat pad and the sum weight of epididymal, retroperitoneal, and mesenteric fat pads was significantly higher in F/ST compared with SU (Table 3).

Glucose and insulin data. Basal glucose, clamp glucose, and clamp insulin levels were not significantly different among groups. Basal insulin levels were significantly increased in SU, F/G, and F/ST compared with ST (Tables 4 and 5).

Glucose kinetics and glucose infusion rate. Basal glucose R_a was not significantly different among ST, SU, and F/ST but was significantly lower in F/G compared with ST. During the clamps, endogenous glucose R_a was significantly higher in the SU, F/G, F/ST compared with ST. Endogenous glucose R_a was significantly higher in F/G compared with SU and F/ST. Insulin suppression of glucose R_a was significantly greater in ST compared with SU, F/G, and F/ST. Insulin suppression of glucose R_a was significantly lower in F/G compared with the SU and F/ST (Table 5).

Glucose R_d during the clamp in SU, F/G, and F/ST was significantly lower then ST. Glucose R_d was significantly lower in F/ST compared with SU and F/G. The glucose infusion rate (GIR) needed to maintain

Table 4. *Preclamp insulin, glucose, and glucose kinetics*

Diet	Insulin, μU/ml	Glucose, mg/dl	R_a, $mg \cdot kg^{-1} \cdot min^{-1}$
ST	32.6 ± 2.5	131.0 ± 0.8	11.5 ± 0.5
SU	50.8 ± 2.2*	135.2 ± 5.1	10.1 ± 0.4
F/G	49.2 ± 5.8*	141.9 ± 5.5	9.0 ± 0.7*
F/ST	56.5 ± 6.3*	145.0 ± 4.0	10.6 ± 0.6

All values are means ± SE ($n = 9$ ST, SU, F/G; $n = 8$ F/ST). Insulin is average plasma insulin levels from 3 samples taken during the final 15 min of the basal period. Glucose is average glucose levels from 3 samples taken during the final 15 min of the basal period. R_a is average glucose rate of appearance from 3 samples taken during the final 15 min of the basal period. *Significantly different from ST ($P < 0.05$).

Table 5. *Clamp glucose kinetics data*

Diet	Clamp-Insulin, μU/ml	Clamp-Glu, mg/dl	GIR	Endo R_a	Clamp R_d	Suppress
				$mg \cdot kg^{-1} \cdot min^{-1}$		
ST	138.6 ± 6.2	128.0 ± 1.2	22.8 ± 1.1	1.9 ± 0.5	24.7 ± 1.2	9.6 ± 0.4
SU	134.5 ± 7.0	128.3 ± 1.9	13.4 ± 0.9*	4.6 ± 0.6*‡	17.9 ± 0.6*	5.6 ± 0.5*‡
F/G	128.5 ± 10.5	127.2 ± 3.0	9.5 ± 1.7*†	6.7 ± 0.8*	16.2 ± 1.3*	2.2 ± 1.2*
F/ST	131.3 ± 10.9	124.3 ± 4.0	11.3 ± 1.3*	4.0 ± 0.7*‡	15.3 ± 1.8*	6.6 ± 0.7*‡

All values are means ± SE (n = 9 ST, SU, F/G; n = 8 F/ST). Clamp-insulin is average of 3 samples taken during the final 15 min (120, 127.5, 135 min) of the clamp. Clamp-Glu is average glucose levels during the final 15 min of the clamp. GIR is time-weighted average of the glucose infusion rate during the final 15 min of the clamp. Endo R_a is average rate of glucose appearance during the final 15 min of the clamp. Clamp R_d is average rate of glucose disappearance during the final 15 min of the clamp. Suppress is average rate of insulin suppression of glucose appearance during the final 15 min of the clamp. *Significantly different from ST ($P < 0.05$). †Significantly different from SU ($P < 0.05$). ‡Significantly different from F/G ($P < 0.05$).

euglycemia was significantly lower in SU, F/G, and F/ST compared with ST. GIR was significantly lower in F/G group compared with SU.

Muscle glycogen synthesis rate and glycogen content. Glycogen concentrations were significantly lower in SU, F/G, and F/ST compared with ST for most of the muscles measured. Tracer estimated muscle glycogen synthesis was significantly lower in SU, F/G, and F/ST compared with ST (Table 6).

Liver triglycerides. Liver triglycerides (μmol/g) were significantly higher ($P < 0.05$) in SU, F/G, and F/ST (10.5 ± 0.9, 9.5 ± 1.0, 10.6 ± 1.3, respectively) compared with ST (6.0 ± 0.4 μmol/g).

DISCUSSION

In this study, IVGTT and euglycemic, hyperinsulinemic clamps were used to compare the effects of sucrose with that of fructose + starch and fructose + glucose on glucose tolerance and whole body insulin action in rats. The results indicate that diets containing high levels of sucrose, fructose and glucose, or fructose and starch significantly impair glucose tolerance as well as hepatic and peripheral insulin action. These data suggest that fructose is the primary nutrient mediator of sucrose-induced glucose intolerance and insulin resistance.

Study 1: IVGTT

Fasting insulin levels in the SU, F/G, and F/ST groups were more than double the levels seen in the ST control group. However, fasting plasma glucose levels were similar among all groups. These data suggest that whole body insulin action was reduced similarly among the three experimental diet groups.

GAUC was significantly higher in the three experimental diet groups compared with the ST control. However, it was not significantly different among the three experimental diet groups. Glucose tolerance is dependent on tissue sensitivity to insulin as well as the circulating levels of this hormone and the effectiveness of glucose to stimulate its own uptake while concomitantly suppressing hepatic glucose production (1). From the present data, it appears that diets containing high levels of sucrose or fructose in combination with glucose or starch all impair glucose tolerance. Results from the euglycemic, hyperinsulinemic clamp (discussed below) demonstrate that insulin action on glucose metabolism was impaired in all three experimental diet groups. However, recent data suggested that the impact of insulin action on glucose tolerance during an IVGTT is minimal in the 24-h-fasted rat (12). In fact, McArthur et al. (12) demonstrated that the glucose response to an IVGTT was primarily a function of glucose effectiveness. Thus the diet-induced reduction in glucose tolerance observed in the present study may involve impairments in "glucose action." Further studies are needed to quantify the independent roles of insulin action and glucose effectiveness on diet-induced glucose intolerance.

It must be noted that the present data underestimate the impairment in glucose tolerance. Twenty of the thirty animals in the experimental groups had not returned to baseline glucose levels after the 80-min IVGTT period (SU, n = 6; F/G, n = 7; F/ST, n = 7). In contrast, only 1 of 10 animals in the control group had not returned to baseline glucose levels after the 80-min IVGTT period. If the experimental period had been

Table 6. *Muscle glycogen content and synthetic rate*

Diet	Soleus	Gastrocnemius	Vastus	Abdominal	Synth Rate, mg/kg/min
	mg/g				
ST	11.0 ± 1.0	12.1 ± 1.4	8.3 ± 1.0	9.3 ± 0.6	11.9 ± 1.3
SU	7.0 ± 0.5*	6.6 ± 0.9*	6.3 ± 0.9	7.3 ± 0.8	6.2 ± 1.2*
F/G	6.4 ± 0.5*	6.4 ± 1.1*	5.1 ± 0.6*	5.9 ± 1.2*	7.3 ± 0.7*
F/ST	4.8 ± 0.6*	5.3 ± .06*	4.7 ± 0.6*	6.5 ± 0.7	5.2 ± 0.6*

All values are means ± SE (n = 9 ST, SU, F/G; n = 8 F/ST). Vastus is vastus lateralis muscle; Abdominal is abdominal wall muscle. Synth Rate is average glycogen synthesis rate in soleus, gastrocnemius, rectus abdominous, and vastus lateralis during clamp. *Significantly different from ST ($P < 0.05$).

longer, the GAUC would have been even higher for the experimental groups.

The IAUC was higher in the experimental groups compared with the ST controls, but significantly so only in the sucrose-fed animals. However, because the insulin response was not significantly different among experimental groups it is unlikely that these diets resulted in different effects on insulin secretion and/or clearance.

Study 2: Euglycemic, Hyperinsulinemic Clamps

The GIR required to maintain euglycemia during the clamp in the SU-, F/G-, and F/ST-fed animals was significantly lower compared with the ST-fed controls. These data indicate that all three experimental diets produced whole body insulin resistance. The lower GIR was due, in part, to muscle insulin resistance, because tracer-estimated muscle glycogen synthesis was significantly lower in the experimental groups compared with starch-fed controls.

Insulin suppression of glucose R_a was significantly lower in SU, F/G, and F/ST compared with ST. Thus, in addition to effects in muscle, the three experimental diets produced hepatic insulin resistance (this assumes that the liver is the primary contributor to glucose R_a). From these data, it is evident that all three diets resulted in both hepatic and peripheral insulin resistance. More importantly, they demonstrate that the fructose moiety of sucrose, which is isolated in the F/ST diet, is the causative agent. These data are in agreement with those of Thorburn et al. (20).

The trigger that links simple sugars and the development of insulin resistance has not been identified. Elevated circulating and tissue triglycerides are often observed in diet-induced insulin resistance (14, 20). Recent work has continued to demonstrate an association between elevated triglyceride concentration and insulin resistance (9, 10). The present data also demonstrate that the three experimental diets resulted in significantly elevated circulating and liver triglyceride concentrations. Whether this elevation contributes to the development of insulin resistance or results from the insulin-resistant state remains uncertain.

There were several subtle but statistically significant differences among the three experimental groups. Basal rates of glucose R_a and insulin suppression of glucose R_a were reduced in the F/G group. Whether this reduction reflects differences in fructose and glucose that originate from the diet versus those that originate from hydrolysis of sucrose is presently unknown.

In animals used to study insulin action, energy intake in F/G and F/ST was significantly greater compared with SU. In contrast, weight gain among these groups was not significantly different. These data suggest that energy expenditure by the F/G and F/ST groups was greater than SU during the 5-wk dietary period resulting in weight gains during the 5-wk dietary period that were not significantly different from SU. The sum of the three fat pads that were weighed was significantly greater in F/ST compared with SU, although body weight after the 5-wk dietary period was not significantly different. Because fat mass can influence insulin action, this difference among the experimental groups must be considered when comparing groups and when comparing these data to other studies. It is unlikely to significantly influence the data as suggested by the results from the IVGTT where energy intake was not different among groups.

In the euglycemic, hyperinsulinemic clamp studies we chose to clamp all animals at similar glucose concentrations (on average 124–128 mg/dl). Because average preclamp glucose levels ranged from 131 to 145 mg/dl, this approach resulted in a greater average decrease in glucose concentration (preclamp minus clamp glucose) in the experimental groups compared with the starch group. This was required to ensure that glucose levels, and thus glucose's ability to mediate its own disposal, were normalized among groups. It is unlikely that this procedure would result in any perturbation (e.g., counterregulation) that could account for the observed reductions in insulin action. This is supported by the fact that in previous studies using a similar methodology, cortisol levels were not different among groups (unpublished observations).

With the use of IVGTT and euglycemic, hyperinsulinemic clamps, the present study compared the effects of high levels of dietary sucrose, fructose and glucose, and fructose and starch on glucose tolerance and whole body insulin action in rats. The results indicate that all three diets significantly impaired glucose tolerance and hepatic and peripheral insulin action. Because fructose in combination with starch was able to elicit a similar pattern and magnitude of insulin resistance and glucose intolerance to that of sucrose and fructose and glucose, these data indicate that fructose is the primary mediator of sucrose-induced insulin resistance and glucose intolerance.

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