

Effects of Dietary Fructose or Glucose on Triglyceride Production and Lipogenic Enzyme Activities in the Liver of Wistar Fatty Rats, an Animal Model of NIDDM

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Abstract. Effects of dietary carbohydrates on triglyceride production and hepatic lipogenic enzyme activities were examined in Wistar fatty rats, an animal model of noninsulin dependent diabetes mellitus, fed fructose or glucose and were compared with those of Wistar lean rats. Carbohydrates were supplied in 10% drinking solutions for 21 days. As compared with lean rats, Wistar fatty rats were characterized by hyperglycemia, hyperinsulinemia and hypertriglyceridemia, the last of which was associated with an increased hepatic activity of fatty acid synthetase and an increased rate of triglyceride secretion from the liver to the circulation. Feeding fructose to genetically obese diabetic rats produced a threefold increase in the hepatic activity of fatty acid synthetase, a twofold increase in NADPH-generating enzymes (malic enzyme and glucose-6-phosphate dehydrogenase) and a 56% increase in the rate of triglyceride secretion, with a resultant 86% increase in plasma triglyceride concentrations. Feeding glucose produced a similar increase in the activity of NADPH-generating enzymes and triglyceride production in the fatty liver but it differed in producing no change in plasma triglyceride concentrations or hepatic fatty acid synthetase activity. Neither dietary fructose nor glucose changed glycemia or insulinemia. These results show that in genetically obese, diabetic rats feeding fructose and glucose is associated with an increase in hepatic lipogenic enzyme activities and triglyceride production, and suggest that fructose stimulates triglyceride production but impairs triglyceride removal, whereas glucose stimulates both of them.

Key words: Carbohydrates, Triglyceride secretion rate, Fatty acid synthetase, Malic enzyme, Glucose-6-phosphate dehydrogenase

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FEEDING sucrose or fructose has long been associated with an increase in plasma triglyceride concentrations in laboratory animals [1]. It is reported that in patients with non-insulin-dependent diabetes mellitus high-carbohydrate

diets caused persistent deterioration of glycemic control and accentuation of hyperinsulinemia as well as increased plasma triglyceride and very-low-density lipoprotein (VLDL) cholesterol [2].

We have previously shown that feeding fructose, but not glucose, into intact male Wistar rats produced an increase in the rate of triglyceride secretion as well as an impairment of triglyceride removal [3]. In addition, infusing exogenous insulin into fructose-fed, but not glucose-fed, male Wistar rats produced a further increase in

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triglyceride production. In contrast, triglyceride concentrations fell to the level of intact male Wistar rats receiving neither sugars nor exogenous insulin [3]. These results suggest that exogenous insulin restores fructose-induced impairment of triglyceride removal but neither fructose nor glucose supplementation produced significant changes in the rate of triglyceride secretion in Zucker fatty rats [4], which were characterized by endogenous hyperinsulinemia and hypertriglyceridemia but normoglycemia [4–6]. The present studies were therefore designed to determine the effects of dietary fructose or glucose on the rate of triglyceride production and the activity of lipogenic enzymes in the liver of Wistar fatty rats, which were characterized by endogenous hyperinsulinemia, hypertriglyceridemia and diabetes mellitus [5].

Materials and Methods

Animals

Three groups of male Wistar fatty rats and one group of male Wistar lean rats, 18 weeks of age, bred at the Central Laboratory of Takeda Chemical Industries, were used throughout the study. They were maintained in individual cages at a constant temperature (23 °C) with a fixed (24-h) artificial light cycle. Two groups of Wistar fatty rats were fed a stock diet (CE-2, Oriental Yeast, Tokyo, Japan) and allowed *ad libitum* access to drinking water that contained either 10% (wt/vol) fructose or glucose for 21 days. The third group of fatty rats and one group of lean rats were given *ad libitum* access to rat chow and drinking water that contained no carbohydrate (chow controls). Time-course changes in plasma concentrations of glucose, insulin, triglyceride and cholesterol were measured in the fed state at the time intervals indicated in Fig. 1.

Triglyceride secretion rates

On the night of day 20, chow was removed but all the rats continued to receive water containing 10% sugar to drink until the end of the experiments. The rate of triglyceride secretion was measured on day 21, between 0900 h and noon, by the Triton

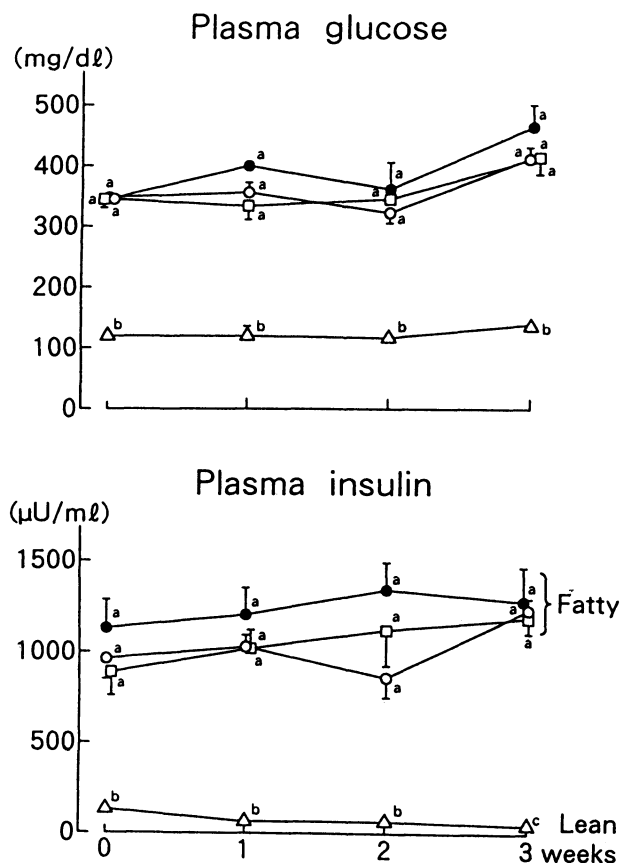


Fig. 1. Time-course changes in postprandial plasma concentrations of glucose and insulin in Wistar fatty rats fed glucose (closed circles) or fructose (squares), and in Wistar fatty and lean rats receiving no carbohydrate (open circles and triangles, respectively). Sugars were supplied in drinking solutions for 3 weeks. Mean \pm SEM for 5 rats in each group. Means with different superscript letters in each variable at the same time period are significantly different at $P < 0.05$.

method. The suitability of this procedure has been previously discussed and fully described by us in Wistar lean rats [7, 8] and by Simonelli and Eaton in Zucker fatty rats [9].

The batch of Triton WR1339 (Nakarai Chemicals, Kyoto, Japan) used in this study has been demonstrated to completely block the removal of VLDL-triglyceride from the circulation and to produce a linear increase in triglyceride concentrations for 90 min. Triton was dissolved in distilled water (300 mg/ml) and injected into the tail vein (600 mg/kg body weight) of rats under light ether anesthesia. Blood was obtained before

and at 45 and 90 min after the injection. The rate of triglyceride production was calculated from the increase in plasma triglyceride levels per minute multiplied by the plasma volume of the rat, and the results were expressed as mg/min [3]. The fractional catabolic rate (FCR) for plasma triglyceride was calculated as the triglyceride secretion rate divided by plasma triglyceride pool size [10].

Immediately after the last blood sampling, an aliquot of each liver was removed under ether anesthesia, homogenized with 3 vol. of 0.25 M sucrose and then centrifuged at 10500 rpm at 4 °C. The supernatant was stored at -80 °C until the assay of enzyme activities. Another aliquot of the liver was immediately frozen to measure lipid content.

Lipogenic enzyme activities

After the supernatant was filtered through a Sephadex G-25 column, fatty acid synthetase activity in the filtrate was assayed according to Hsu [11], malic enzyme according to Ochoa [12] and glucose-6-phosphate dehydrogenase according to Glock and McLean [13]. The enzyme activities in the supernatant of liver homogenates are expressed as mU/mg protein, when 1 mU is the amount catalyzing the formation of nmol product/min at 37 °C for fatty acid synthetase and the utilization of NADP for malic enzyme and glucose-6-phosphate dehydrogenase.

Determinations

All blood samples were centrifuged at 4 °C and plasma was stored at -20 °C until assayed. Plasma glucose, triglyceride and cholesterol were determined enzymatically with an Encore (Backer Instruments, Allentown, PA). Plasma insulin was assayed by the double-antibody method with commercially available kits (Shionoria®, Shionogi, Tokyo, Japan). The concentration of triglyceride, cholesterol and phospholipid in the supernatant of liver homogenates was measured by respective enzymatic methods with commercially available kits (Iatron Laboratories, Tokyo, Japan) after lipid extraction with chloroform-methanol [14].

Statistical analysis

This was carried out by Duncan's multiple range test [15]. Data are expressed as the means \pm SEM.

Results

There was no difference in the final body weight or in epididymal adipose tissue weight of the three groups of fatty rats, although two groups of rats fed sugars had a greater total caloric intake (Table 1). Fatty rats consumed more glucose than fructose.

As previously reported [5, 16, 17], Wistar fatty rats were characterized by hyperglycemia, hyperinsulinemia and hypertriglyceridemia (Figs. 1 and 2). Neither fructose nor glucose supplementation produced significant changes in postprandial plasma levels of glucose and insulin in fatty rats (Fig. 1). Feeding fructose to fatty rats produced an increase in postprandial plasma triglyceride levels, which occurred at week 1 and continued throughout the experiment. In contrast, there was no change in triglyceride levels in fatty rats fed glucose.

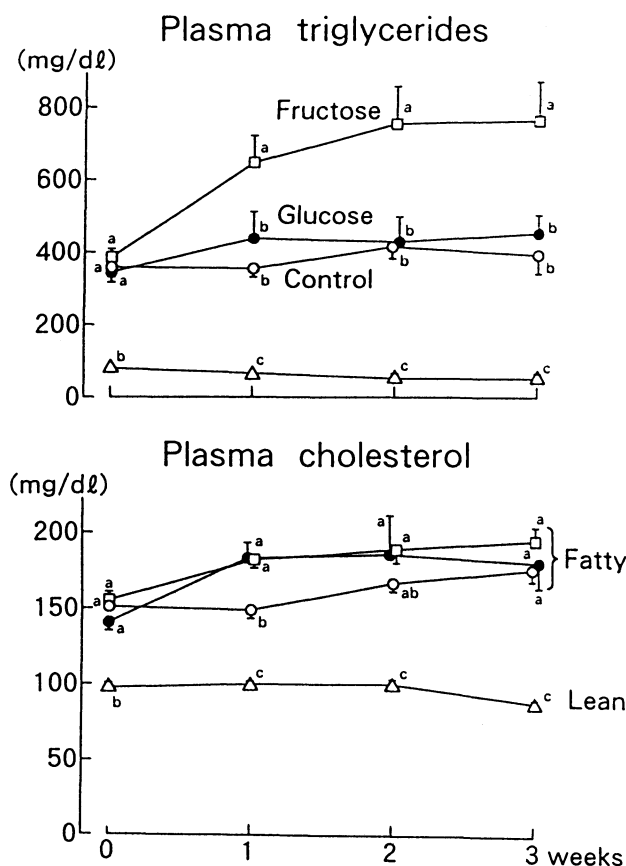
As compared with lean rats, Wistar fatty control rats had a 6-fold increase in fasting plasma triglyceride concentrations associated with a 3-fold increase in the rate of triglyceride production (Fig. 3). Feeding fructose to Wistar fatty rats for 21 days doubled fasting plasma triglyceride concentrations (Fig. 3). This was associated with an increase of 56% in the rate of triglyceride production. Feeding glucose was also associated with a 43% increase in the triglyceride production rate. Despite this, fasting plasma triglyceride concentrations in glucose-fed fatty rats were similar to those of fatty rats receiving no carbohydrate. The estimated FCR was higher in glucose-fed fatty rats ($0.041 \pm 0.008 \text{ min}^{-1}$) than in fructose-fed rats or fatty controls (0.020 ± 0.002 and $0.025 \pm 0.001 \text{ min}^{-1}$, respectively, $P < 0.05$) but still lower than in lean controls ($0.055 \pm 0.005 \text{ min}^{-1}$, $P < 0.05$).

As compared with lean livers, fatty control livers contained more triglyceride and cholesterol but less phospholipid (Table 1). The activity of fatty acid synthetase and NADPH-generating enzymes (malic enzyme and glucose-6-phosphate dehydrogenase) was higher in fatty control livers than in lean

Table 1. Body weight, carolic intake, liver weight and hepatic lipid content

Phenotype	lean	fatty	fatty	fatty
Carbohydrate supplemented	none	none	glucose ⁺	fructose ⁺
Body weight (g)				
Starting	431 ± 7 ^a	593 ± 13 ^b	588 ± 18 ^b	586 ± 12 ^b
Final	432 ± 9 ^a	625 ± 12 ^b	626 ± 16 ^b	635 ± 13 ^b
Epididymal adipose tissue (g)	4.9 ± 0.2 ^a	17.9 ± 0.9 ^b	17.4 ± 1.2 ^b	18.2 ± 0.6 ^b
Caloric intake (cal/day)*				
Total	76 ± 3 ^a	126 ± 4 ^b	153 ± 6 ^c	140 ± 5 ^c
From chow	76 ± 3 ^a	126 ± 4 ^b	90 ± 3 ^c	100 ± 2 ^d
From sugar	none	none	63 ± 4 ^a	39 ± 4 ^b
Liver weight (g)	9.6 ± 0.6 ^a	21.8 ± 0.7 ^b	26.0 ± 1.3 ^c	28.6 ± 1.2 ^c
Hepatic lipid content (mg/g)				
Triglycerides	11.8 ± 1.0 ^a	74.0 ± 6.9 ^b	97.4 ± 17.3 ^b	69.1 ± 4.3 ^b
Cholesterol	2.9 ± 0.1 ^a	4.9 ± 0.7 ^b	3.9 ± 0.6 ^{ab}	3.2 ± 0.4 ^a
Phospholipids	30.4 ± 1.1 ^a	23.0 ± 0.8 ^b	18.8 ± 0.7 ^c	19.0 ± 0.3 ^c

Mean ± SEM for 5 rats in each group. ⁺Carbohydrates were supplied in 10% drinking solution for 21 days. *Calculated for a stock diet (3.45 cal/g) and sugars (4.0 cal/g) consumed. Means not sharing a common letter in the same row are significantly different from each other at $P < 0.05$ or less.



control liver (Table 2), although the difference between the two groups in the activity of NADPH-generating enzymes did not reach statistical significance. Either fructose or glucose supplementation produced a decrease in cholesterol and phospholipid content but there was no significant difference in hepatic triglyceride content among the three groups of fatty rats. The activity of fatty acid synthetase tripled in response to fructose but not to glucose. In contrast, the activity of NADPH-generating enzymes increased to the same extent in response to either fructose or glucose feeding.

Discussion

The present studies have shown that hypertriglyceridemia in Wistar fatty rats was in part a metabolic consequence of an increased rate

Fig. 2. Changes in postprandial plasma levels of triglyceride and cholesterol in response to feeding fructose or glucose in Wistar fatty rats. See the legend to Fig. 1 for rat groups and statistical significance. Mean ± SEM for 5 rats in each group.

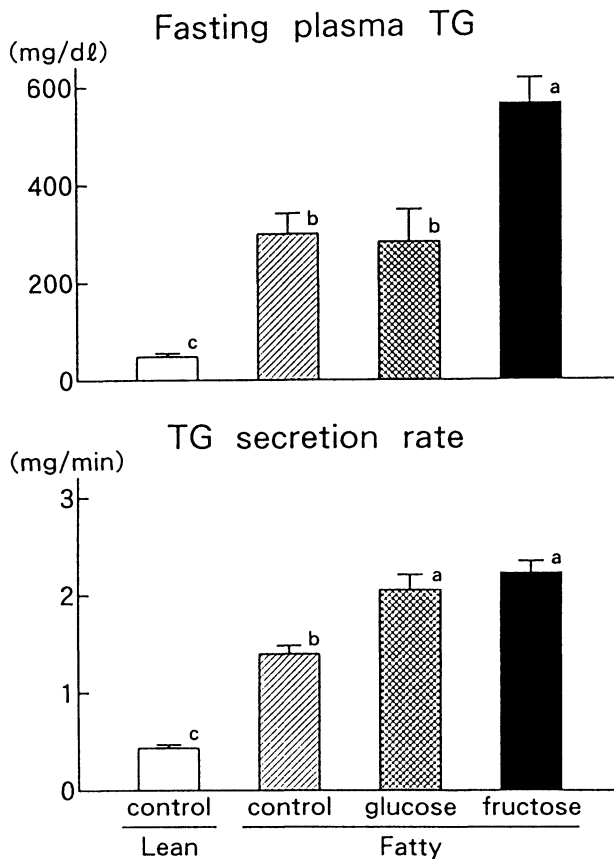


Fig. 3. Fasting plasma triglyceride (TG) concentrations and TG secretion rate in Wistar fatty rats fed glucose or fructose, and in Wistar fatty and lean rats receiving no carbohydrate (controls). All rats received indicated sugar *ad libitum* as a 10% solution in place of drinking water. Bars with different superscript letters are significantly different from each other at $P < 0.05$. Mean \pm SEM for 5 rats in each group.

of triglyceride secretion from the liver. This was associated with greater hepatic activities of fatty acid synthetase and NADPH-generating enzymes, as previously reported [18, 19]. In the present study, the rate of triglyceride secretion averaged $16.2 \mu\text{mol}/\text{min}$ in Wistar fatty control rats and this figure was comparable to the rate of triglyceride secretion in Zucker fatty rats [4, 8]. The Triton method is reproducible and reliable when it is used for comparison between different diets or genotypes, although it probably does not provide absolute values for the rate of lipoprotein production.

We have previously shown that neither fructose nor glucose supplementation produced significant changes in the rate of triglyceride secretion in Zucker fatty rats [4]. Since the previous study using the perfused liver [20] demonstrated that a major portion of the increased triglyceride secretion by the livers from Zucker fatty rats was derived from endogenous rather than exogenous sources, it is likely, as we previously suggested [4], that fructose is not readily converted to fat in Zucker fatty rat livers. In contrast to Zucker fatty rats, in Wistar fatty rats, both dietary fructose and glucose stimulated triglyceride production in the present studies. It is reported that as compared with Zucker fatty rats, Wistar fatty rats were less hypertriglyceridemic and had lower hepatic activities of lipogenic enzymes [5, 6]. For this reason, the observation that feeding fructose or glucose stimulated the activity of malic enzyme and glucose-6-phosphate dehydrogenase in the livers of Wistar fatty rats may be associated with the stimulation of triglyceride production in these

Table 2. The activity (mU/mg protein) of lipogenic enzymes in the liver

Phenotype	lean	fatty	fatty	fatty
Carbohydrate-supplemented	none	none	glucose ⁺	fructose ⁺
Fatty acid synthetase	0.04 ± 0.00^a	0.28 ± 0.03^b	0.36 ± 0.11^b	0.86 ± 0.07^c
Malic enzyme	8.87 ± 0.87^a	13.47 ± 1.63^a	22.40 ± 1.02^b	27.16 ± 2.98^b
Glucose-6-phosphate dehydrogenase	23.0 ± 1.8^a	33.2 ± 4.3^a	53.0 ± 3.0^b	55.2 ± 6.8^b

Mean \pm SEM for 5 rats in each group. ⁺Carbohydrates were supplied in 10% drinking solutions for 21 days. Means not sharing a common letter in the same row are significantly different from each other at $P < 0.05$ or less.

rats. However, triglyceride contents in livers of fatty rats fed fructose or glucose did not differ from those in fatty control livers.

Plasma triglyceride concentrations are reflected by the balance between the production and removal of triglyceride-rich lipoproteins in the circulation. In the present studies, glucose stimulated triglyceride production by 43% in Wistar fatty rats. Despite this, triglyceride concentrations remained unchanged, suggesting that glucose stimulated triglyceride removal. This was supported by greater FCR in glucose-fed fatty rats as compared with fatty control rats in the present studies and may be consistent with the observation by Hirano *et al.* [21] that the FCR of VLDL-triglyceride, measured by a tracer method employing radioactive glycerol, was higher in male Wistar rats fed glucose for 14 days than in controls. In contrast to glucose, feeding fructose to fatty rats produced an 86% increase in triglyceride concentrations associated with a 56% increase in triglyceride production, suggesting catabolic defects in VLDL-triglyceride in fructose-fed fatty rats. This may be consistent with the observation that fructose-induced hypertriglyceridemia may in part result from an impairment in the ability of intact rats fed fructose to hydrolyze VLDL-triglyceride, and of their livers to remove VLDL-triglyceride [22].

Changes in VLDL that make them less susceptible to removal may be associated with the lower ratio of apolipoprotein E to C found in fructose-fed rats [23].

In the present studies, fructose, but not glucose feeding, produced a further increase in fatty acid synthetase activity in livers of Wistar fatty rats, as previously reported in lean intact rats [24, 25]. Glucose, however, stimulated hepatic triglyceride production as well as the activity of enzymes supplying NADPH (glucose-6 phosphate dehydrogenase and malic enzyme) to the same extent as fructose. These results suggest that the two enzymes supplying NADPH may be more closely related to the secretion of triglyceride from the liver than fatty acid synthetase. Since Wistar fatty rats had greater hepatic fatty acid synthetase activity than did lean controls, as previously reported [18, 19] and confirmed in the present studies, livers of Wistar fatty rats may be able to produce more triglyceride in response to glucose in the face of no further increase in the activity of fatty acid synthetase. No difference between fatty controls and sugar supplemented groups in liver triglyceride content, despite an increase in hepatic lipogenesis, may be consistent with previous observations [26, 27].

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