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SHORT REPORT

High D(+)-Fructose Diet Adversely Affects Testicular Weight Gain in Weaning Rats—Protection by Moderate D(+)-Glucose Diet

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Abstract: The use of high D(+)-fructose corn syrup has increased over the past several decades in the developed countries, while overweight and obesity rates and the related diseases have risen dramatically. However, we found that feeding a high D(+)-fructose diet (80% D(+)-fructose as part of the diet) to weaning rats for 21 days led to reduced food intake (50% less, $P < 0.0001$) and thus delayed the weight gains in the body (40% less, $P < 0.0001$) and testes (40% less, $P < 0.0001$) compared to the no D(+)-fructose diet. We also challenged a minimum requirement of dietary D(+)-glucose for preventing the adverse effects of D(+)-fructose, such as lower food intake and reduction of body weight and testicular weight; the minimum requirement of D(+)-glucose was ≈23% of the diet. This glucose amount may be the minimum requirement of exogenous glucose for reducing weight gain.

Keywords: D(+)-fructose, D(+)-glucose, testis, diet, rat

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Introduction

In developed countries, consumption of soft drinks has increased the last three decades and is partly responsible for the increase in serum levels of free fatty acids, triglycerides and cholesterol,[1] obesity,[2,3] metabolic syndrome, and hypertension.[4] Soft drinks, originally sweetened with sucrose, are now sweetened by other caloric sweeteners, such as D(+)-fructose. Some studies show positive correlations between intake of sweeteners and body weight,[5–7] whereas others show inverse correlations.[8–10] Thus, the effect of sweeteners on body weight remains unclear. Some experts have implicated high-D(+)-fructose corn syrup as a possible contributing factor to weight gain, and thus the rise in the prevalence of obesity.[2,5,11] In this study, we investigated the short-term effect of high D(+)-fructose diet on food intake, body weight, and organ weights in weaning rats.

Dietary D(+)-glucose is the sole energy source in the central nervous system, testis, renal tubule, erythrocyte, and some tissues. However, D(+)-glucose is not an indispensable nutrient because D(+)-glucose can be biosynthesized from other nutrients such as amino acids and D(+)-fructose. Nevertheless, the intake of D(+)-glucose or its homopolymer such as starch is very important for maintaining good health. Thus, we investigated the requirement of dietary D(+)-glucose for preventing D(+)-fructose toxicity.

Methods

Chemicals

Vitamin-free milk casein, L-methionine, D(+)-fructose, and D(+)-glucose were purchased from Wako Pure Chemical Industries (Osaka, Japan). Corn oil was obtained from Ajinomoto (Tokyo, Japan). A mineral mixture (AIN-93-G-MX)[12] and vitamin mixture (AIN-93-VX)[12] were purchased from Oriental Yeast Kogyo (Tokyo, Japan). All other chemicals were the highest purity available from commercial sources.

Animals and diets

The care and treatment of the experimental animals conformed with The University of Shiga Prefecture guidelines for the ethical treatment of laboratory animals. The room temperature was maintained at approximately 22°C and 60% humidity with a 12-h/12-h light/dark cycle (06:00–18:00/18:00–06:00).

Weaning male rats of the Wistar strain (3 weeks old, approximately 35 g) were obtained from Clea Japan (Tokyo, Japan) in all experiments and immediately placed in individual metabolic cages (CT-10; Clea Japan). The compositions of the diets are shown in Table 1. Rats had access to food and water ad libitum.

In experiment 1, the weaning male rats were divided into four groups (n = 5, each group); group 1 was fed a low-protein diet high in D(+)-glucose (10% casein-80% D(+)-glucose diet), group 2 was fed a low-protein diet high in D(+)-fructose (10% casein-80% D(+)-fructose diet), group 3 was fed an ordinary protein diet high in D(+)-glucose (20% casein-70% D(+)-glucose diet), and group 4 was fed an ordinary protein diet high in fructose (20% casein-70% D(+)-glucose diet) for 21 days in order to identify the most sensitive tissue in terms of weight. Body weight and food intake were measured daily at approximately 09:00. Rats were sacrificed by decapitation and the liver, kidneys, testes, and brain were dissected and weighed.

In experiment 2, the weaning male rats were divided into five groups (n = 5, each group); group 1 was fed an ordinary protein diet high in D(+)-fructose (10% casein-70% D(+)-fructose diet) and groups 2–5 were fed an ordinary protein diet with varying amounts of D(+)-fructose and D(+)-glucose for 21 days. We chose a diet containing 35% D(+)-glucose and 35% D(+)-fructose as a control diet in the present experiment. The composition of the 20% casein-based 35% D(+)-glucose and 35% D(+)-fructose diet is equivalent to the AIN-93G diet for a short time. This is because food intake and body weight gains between weaning fed the diet containing 20% casein, 0.2% L-methionine, 46.9% gelatinized corn starch, 23.4% corn starch, 23.4% sucrose, 5% corn oil, 3.5% AIN-93G mineral mixture, and 1% AIN-93 vitamin mixture (referred to as the modified AIN-93G diet[13–15] and weaning rats fed a diet consisting of 20% casein and 70% sucrose were nearly the same. The diet containing only sucrose as the carbohydrate source can maintain body weight and food intake with the modified AIN-93G diet. For other diets, the amounts of glucose were appropriately reduced. Body weight and food intake were measured daily at approximately 09:00.

The rats were sacrificed by decapitation. Blood samples were taken into a Venoject II (VP-DK052K; Terumo Corporation, Tokyo, Japan) and tissue samples were collected. The liver, kidneys, brain, and testes

Table 1. Compositions of the diets.

	10% Casein-based diet (Experiment 1)		20% Casein-based diet (Experiment 1)	
	Control	**Test**	**Control**	**Test**
	80% D(+)-glucose + 0% D(+)-fructose	**0% D(+)-glucose + 80% D(+)-fructose**	**70% D(+)-glucose + 0% D(+)-fructose**	**0% D(+)-glucose + 70% D(+)-fructose**
	%	**%**	**%**	**%**
Vitamin-free milk casein	10	10	20	20
L-Methionine	0.1	0.1	0.2	0.2
D(+)-Glucose	80.4	0	70.3	0
D(+)-Fructose	0	80.4	0	70.3
Corn oil	5	5	5	5
Mineral mixture (AIN-93-G)*	3.5	3.5	3.5	3.5
Vitamin mixture (AIN-93)*	1	1	1	1

	20% Casein-based diet (Experiment 2)				
	Test group				**Control group**
	0% D(+)-glucose + 70% D(+)-fructose	**14% D(+)-glucose + 56% D(+)-fructose**	**23% D(+)-glucose + 46% D(+)-fructose**	**30% D(+)-glucose + 40% D(+)-fructose**	**35% D(+)-glucose + 35% D(+)-fructose**
	%	**%**	**%**	**%**	**%**
Vitamin-free milk casein	20	20	20	20	20
L-Methionine	0.2	0.2	0.2	0.2	0.2
D(+)-Glucose	0	14.1	23.4	30.1	35.2
D(+)-Fructose	70.3	56.2	46.9	40.2	35.2
Corn oil	5	5	5	5	5
Mineral mixture (AIN-93-G)*	3.5	3.5	3.5	3.5	3.5
Vitamin mixture (AIN-93)*	1	1	1	1	1

Note: *Reeves RG. Components of the AIN-93 diets as improvements in the AIN-76A diet. *J Nutr*. 1998;127:838S–41S.

were dissected and weighed. The collected blood samples were centrifuged for 10 min at 1500 × *g* to separate plasma. The resulting plasma were retained and stored −80°C until analysis.

General biomarkers in blood

Enzyme activities of aspartate transaminase (AST) and alanine transaminase (ALT) were analyzed using Fuji Dry-Chem slide GOT/AST-PIII and Fuji Dry-Chem Slide GPT/ALT-PIII (UV-kinetic, The Japanese Society of Clinical Chemistry transferable method), respectively, using the automated analysis system Fuji Dry-Chem 4000 (Tokyo, Japan). The plasma concentrations of triglycerides (TG), D(+)-glucose, total protein, and total cholesterol were analyzed using Dry-Chem Slides TG-PIII, GLU-PIII, TP-PII, and TCHO-PIII (selective elimination method, direct method), respectively, using the Dry-Chem 4000 (Fuji). Plasma non-esterified fatty acids (NEFA) and total ketone bodies were measured using an enzymatic colorimetric kit (NEFAC-TEST, Wako) and cyclic enzymatic kit (T-KB, Wako), respectively.

Statistical analysis

For statistical evaluation, the significance of the differences in the mean values between the control and each of the test groups was determine using Student's two-tailed t-test for experiment 1. For experiment 2, the significance of the difference in the mean among groups was treated with one-way ANOVA and followed by Bonferroni's post-hoc analysis to compare groups. Differences were considered significant when $P < 0.05$. GraphPad Prism (version 5.00; GraphPad Software, Inc, San Diego, CA, USA) was used for statistical analysis.

Results and Discussion

The influence of high D(+)-fructose is considered to be higher under low-protein conditions.[16,17] Accordingly, we attempted to determine which organ is the most sensitive to a high D(+)-fructose diet in experiment 1. At the 10% protein level, feeding of high D(+)-fructose (0% D(+)-glucose + 80% D(+)-fructose) diet caused extremely low body weight gain concomitant with low food intake compared to those in rats fed D(+)-glucose (80% D(+)-glucose + 0% D(+)-fructose) diet (Table 2). When weaning rats were fed diets containing normal levels of protein (20% casein-based diet), the testicular weight was also lower on the D(+)-fructose diet than in the D(+)-glucose diet (Table 2). Significantly low food intake when on the high D(+)-fructose revealed that unlike D(+)-glucose, D(+)-fructose does not stimulate insulin secretion from pancreatic β-cells.[18] Insulin may be a key element in the chain of events that leads to increased food intake. Of the organ weights, the testes were the most sensitive, which were reduced by 37% (Table 2).

Next, in experiment 2, the amounts of dietary D(+)-glucose were gradually increased under normal protein levels (20% casein as part of the diet). As a control diet, the diet containing 20% casein, 35% D(+)-glucose, 35% D(+)-fructose, and 5% corn oil was used. The test diets contained increasing amounts of dietary D(+)-fructose, including 40%, 46%, 56%, and 70% as part of the diets concomitant with reducing the amounts of D(+)-glucose, including 35%, 30%, 23%, and 14% as part of the diet. Of the test groups, significant differences were observed in the rats fed the 56% D(+)-fructose + 14% D(+)-glucose diet; higher TG, NEFA, and total ketones in the plasma were observed compared to the control group (Table 3). Insulin-mediated D(+)-glucose uptake and metabolism in adipose tissues is thought to play a key regulatory role in leptin concentrations[19,20] because secretion of leptin has been shown to be regulated by insulin.[21] Leptin acts potentially by inhibiting the effects of the orexigenic hormone ghrelin.[22–24] Accordingly, in the case of high D(+)-fructose diets, D(+)-fructose does not

Table 2. Comparison of dietary D(+)-glucose *versus* D(+)-fructose on the food intake, the weights of body and organs in weaning rats fed a low protein and ordinary protein diets (Experiment 1).

	10% Casein-based diet (Experiment 1)		**20% Casein-based diet (Experiment 1)**	
	Control	**Test**	**Control**	**Test**
	80% D(+)-glucose + 0% D(+)-fructose	**0% D(+)-glucose + 80% D(+)-fructose**	**70% D(+)-glucose + 0% D(+)-fructose**	**0% D(+)-glucose + 70% D(+)-fructose**
Body weight gain (g/21 d)	79.8 ± 3.1	28.5 ± 12.6* (36%)	128.4 ± 3.4	96.3 ± 0.8* (75%)
Food intake (g/21 d)	232 ± 10	106 ± 13* (46%)	290 ± 20	201 ± 23* (69%)
Organ weights				
Liver (g)	5.49 ± 0.31	4.24 ± 0.51* (77%)	7.85 ± 0.33	9.20 ± 0.52* (117%)
Kidneys (g)	0.86 ± 0.03	0.69 ± 0.04* (80%)	1.46 ± 0.04	1.42 ± 0.12 (97%)
Testes (g)	1.56 ± 0.04	0.57 ± 0.08* (37%)	1.63 ± 0.12	1.32 ± 0.10* (81%)
Brain (g)	1.15 ± 0.01	1.06 ± 0.03* (92%)	1.25 ± 0.02	1.18 ± 0.02 (94%)

Values are means ± SEM, n = 5.
*Significant difference from the control group in the 10% protein group as calculated by Student's *t*-test ($p < 0.05$).
Parenthesis numbers are percent against the control group.

Table 3. The amounts of dietary D(+)-glucose that can prevent the adverse effects of high D(+)-fructose diet in weaning rats (Experiment 2).

	20% Casein-based diet				
	Test group				Control group
Group name	**70% D(+)-fructose + 0% D(+)-glucose**	**56% D(+)-fructose + 14% D(+)-glucose**	**46% D(+)-fructose + 23% D(+)-glucose**	**40% D(+)-fructose + 30% D(+)-glucose**	**35% D(+)-fructose + 35% D(+)-glucose**
Body weight gain (g/21 d)	93 ± 2[b]	99 ± 3[a]	102 ± 2[a]	102 ± 1[a]	103 ± 2[a]
Food intake (g/21 d)	179 ± 4[b]	187 ± 6[b]	204 ± 2[a]	205 ± 2[a]	209 ± 4[a]
Organ weight					
Testes (g)	1.44 ± 0.02[b]	1.43 ± 0.06[b]	1.52 ± 0.04[a]	1.54 ± 0.04[a]	1.62 ± 0.02[a]
Biological index in plasma					
D(+)-glucose (mg/dL)	78.0 ± 6.3[b]	100.7 ± 5.2[a]	96.2 ± 3.5[a]	107.4 ± 3.7[a]	109.8 ± 3.9[a]
TG (mg/dL)	243.6 ± 23.0[a]	153.4 ± 21.5[b]	166.4 ± 12.2[b]	149.8 ± 11.6[b]	160.7 ± 22.0[b]
NEFA (mEq/L)	11.63 ± 0.19[a]	0.71 ± 0.12[b]	0.72 ± 0.12[b]	0.60 ± 0.12[b]	0.65 ± 0.12[b]
8-Total ketone bodies (μmol/L)	53.9 ± 5.7[a]	29.1 ± 5.3[b]	21.3 ± 4.4[b]	23.6 ± 2.8[b]	25.6 ± 4.5[b]

Values are means ± SEM, n = 5.
One-way ANOVA followed by Benferroni's post hoc test was used to analyze statistical differences among all groups.
[a,b]Labeled means in a row without a common letter differ $p < 0.05$.

stimulate insulin secretion, and this chain of satiety-producing events does not occur. For organ weights, the liver, kidneys, and brain weights did not differ among groups; the weights were approximately 9 g, 1.5 g, and 1.2 g, respectively. Testicular weight was lower in groups consuming the 56% D(+)-fructose + 14% D(+)-glucose and the 70% D(+)-fructose + 0% D(+)-glucose diets compared to the groups consuming the 40% D(+)-fructose + 30% D(+)-glucose and 46% D(+)-fructose + 23% D(+)-glucose and control diets (Table 3). Of the plasma indices for nutritional statuses, total protein, ALT, AST, and total cholesterol concentrations did not show differences among groups; levels were 6 g/dL, 35 U/L, 12 U/L, and 90 mg/dL, respectively. TG, NEFA, and total ketone body concentrations were approximately 2.5, 1.2, and 1.5 times higher in the 70% D(+)-fructose + 0% D(+)-glucose diet group compared to the other groups (Table 3). These results indicate that fatty acid synthesis following D(+)-fructose consumption was more rapid in the liver of rats fed the D(+)-fructose–rich diet than in rats fed the D(+)-glucose-containing diet.[16,17] However, the body weight gain was lower in the 70% D(+)-fructose + 0% D(+)-glucose and the 56% D(+)-fructose + 14% D(+)-glucose groups than in the other groups (46% D(+)-fructose + 23% D(+)-glucose, 40% D(+)-fructose + 30% D(+)-glucose, and control). These results indicate that D(+)-fructose itself does not cause obesity, but it elevates the accumulation of plasma lipids, at least during our short-term experiment.

In summary, we found that feeding of a high D(+)-fructose diet led to delayed testicular weight gain of weaning rats, while a moderate D(+)-glucose diet was able to prevent this. Thus, since high D(+)-fructose diets can cause infertility, further studies are needed to verify these results and the safety of high D(+)-fructose diets. In addition, we found that the minimum requirement for D(+)-glucose for preventing delayed testicular weight gain of weaning rats was 23% compared to rats fed the AIN-93G diet in previous studies.[12–15] This glucose may indicate the minimum requirement for exogenous glucose for weaning rats.

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Author Contributions

Designed the study: KS and TF. Drafted the manuscript: KS. Reviewed the manuscript: TF. Performed the experiment: KS and TF. All authors reviewed and approved the final manuscript.

Competing Interests

The authors disclose no potential conflicts of interest.

Disclosures and Ethics

As a requirement of publication the authors have provided signed confirmation of their compliance with ethical and legal obligations including but not limited to compliance with ICMJE authorship and competing interests guidelines, that the article is neither under consideration for publication nor published elsewhere, of their compliance with legal and ethical guidelines concerning human and animal research participants (if applicable), and that permission has been obtained for reproduction of any copyrighted material. This article was subject to blind, independent, expert peer review. The reviewers reported no competing interests.

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