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Original Article

Effects of excessive fructose on embryo-fetal development in rats

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ABSTRACT — Fructose, a simple carbohydrate, is contained in fruits and honey. It is widely used as a commercial sweetener for beverages and foods and the average fructose intake in the United States was reported to be 49 g/day from 1999 to 2004. It has been reported that excessive intake of fructose induces developmental disorders of the offspring in rats, particularly abnormal glucose and lipid metabolism and neurodevelopment. However, it has not been reported whether excessive intake of fructose induces congenital morphological abnormalities in fetuses. Therefore, we investigated whether an excessive intake of fructose during pregnancy induces congenital morphological abnormalities in the fetus in rats. Pregnant rats were fed a diet containing 13 g, 26 g, 39 g and 65 g fructose / 66 g carbohydrate in 100 g diet, respectively, for the 13% to 65% Fru diet groups (equivalent to about 500, 1000, 1500 and 2500 g/man/day in humans) or control diet from the day after mating until the end of gestation. Caesarean section was performed on gestation day 20, and the fetuses were examined for caesarean section parameters (fetal viability, fetal body weight, placental weight) and fetal external, skeletal and visceral abnormalities. There were no changes/findings in the caesarean section parameters or fetal morphology in any of the fructose diet groups. In conclusion, excessive intake of fructose during gestation did not induce congenital morphological abnormalities in rat fetuses and did not affect fetal viability or development.

Key words: SD rats, Fructose, Caesarean section parameters, Fetal morphology, Commercial sweetener

INTRODUCTION

Fructose (Fru), a simple carbohydrate, is contained in several natural foods including fruits, and honey. Fru is widely used as a commercial sweetener for beverages and foods because of its lower cost and stronger sweetness. In the United States, the average Fru intake was reported to be 49 g/day from 1999 to 2004 (Marriott *et al.*, 2009). In addition, a daily Fru intake of more than 50 g has been reported to increase plasma triglyceride levels (Tappy *et al.*, 2018) and excessive Fru intake associated with a low energy turnover lead to a chronic overproduction of intrahepatic trioses-phosphate, which is secondarily responsible for the development of hepatic insulin resistance, intrahepatic fat accumulation, and increased blood triglyceride concentrations (Tappy, 2018). Although excessive Fru intake alters glucose-lipid metabolism and affects human health, little is known about the effects of Fru on both fetal and maternal health in humans (Thompson and DeBosch, 2021). On the contrary, excessive intake of artificially sweetened and sugar-sweetened beverages is reported to be associated with an increased risk for preterm delivery in humans (Englund-Ögge *et al.*, 2012).

In experimental animals, effects of excessive Fru intake in pregnant rats on the dams and offspring were

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reported (Thompson and DeBosch, 2021). Homeostasis of glucose metabolism is compromised during pregnancy in rats and excessive Fru intake was reported to induce fatty liver and glucose intolerance in pregnant and lactating rats but not in non-pregnant animals (Zou *et al.*, 2012). Fru intake before mating or during pregnancy and lactation was reported to cause low plasma insulin levels (Vickers *et al.*, 2011) and neurodevelopmental disorders in the offspring (Erbas *et al.*, 2018). Similarly, as nutritional and metabolic changes, an increase in food consumption, hyperglycemia, hyperlipidemia and metabolic syndrome have been noted in offspring of mice treated with Fru during pregnancy and lactation (Magenis *et al.*, 2022). Furthermore, excessive Fru intakes was reported to decrease pregnancy rates and litter sizes in mice (Saben *et al.*, 2016). These reports in rats and mice suggest that excessive Fru intake during pregnancy may cause congenital anomalies in the fetus. However, it has not been reported whether excessive intake of Fru induces congenital anomalies in rat fetuses. Therefore, we investigated whether excessive intake of Fru during pregnancy affects embryo-fetal development in rats.

MATERIALS AND METHODS

Diets

The following 5 types of purified pelleted diets were purchased from Research Diet Inc. (New Jersey, USA): D11708C (basal diet modified from AIN-76A, containing 66 g carbohydrate in a 100 g diet) for the control diet group (Group 1), D18071207, D18071206, D18071205 and D11707R (containing 13 g, 26 g, 39 g and 65 g Fru / 66 g carbohydrate in a 100 g diet, respectively) for the 13% to 65% Fru diet groups (Groups 2 to 5). The nutritional information of each diet used in this study is listed in Table 1.

Animals

Fifty copulated female Crl:SD (SD) rats at 8 or 9 weeks of age were obtained from Jackson Laboratory Japan Inc. (Kanagawa, Japan) on day1 of gestation (GD 1). The day of confirmation of copulation was designated as GD 0. The copulated female rats were housed individually in wire-mesh cages kept in an air-conditioned room with a 12-hr light-dark cycle (lighting from 7:00 a.m. to 7:00 p.m.), a temperature of 23 ± 3°C, a relative humidity from 30 to 75% and a ventilation rate of about 15 times per hour. The animals were divided into 5 groups (n=10/group) based on their body weights on GD 1 and then assigned to Group 1 (0% Fru), 2 (13% Fru), 3 (26% Fru), 4 (39% Fru) and 5 (65% Fru). Each Fru diet was fed to the animals *ad libitum* from GD 1 to the day of caesarean section (GD 20). Tap water were available for drinking to the animals *ad libitum*. Experimental procedures for all animals were approved by the Institutional Animal Care and Use Committee of the Toxicology Research Laboratories, Central Pharmaceutical Research Institute, Japan Tobacco Inc. This study was conducted in accordance with the Japanese Law for the Humane Treatment and Management of Animals (Law No. 105, as revised in 2013, issued in October 1, 1973).

Observations, measurements and examinations

Clinical Observations, Measurements of Body Weights and Food Consumption

The animals were observed carefully for any clinical signs once daily from GDs 1 to 20. The animals were weighed and the food consumption per animal was calculated on GDs 1, 3, 5, 7, 9, 11, 13, 15, 17 and 20.

Caesarean Section

On GD 20, all the animals were euthanized by exsanguination from the abdominal aorta under isoflurane anesthesia. The thoracic and abdominal organs were examined in detail for gross lesions. The liver and kidney were weighted after the macroscopic examination and the relative weights of the liver and kidney to the final body weight was calculated. The final body weight was calculated by subtracting the weights of intrauterine contents from the body weights on GD 20, and the weights of the intrauterine contents were calculated from the weights of the gravid uterus and the weights of the uterus. The following data were obtained at the caesarean section: the number of corpora lutea, implantations, live fetuses, dead embryos (early deaths as implantation sites and placental remnants), dead fetuses (late deaths as macerated fetuses and dead fetuses) and the total number of dead embryos and fetuses. The following indices were calculated based on the above data: pre-implantation loss, implantation index, early resorptions (percentage of early deaths), late resorptions, post-implantation loss. These parameters were examined according to the ICH S5 guideline.

Fetal Examinations

All live fetuses were anesthetized with physiological saline and examined for external appearances and in the oral cavities and sexed. The fetal body weights were measured individually. The placentae of the live fetuses were examined for gross lesions and then weighed individually. After the above examinations, approximately one-half of the live fetuses in each litter were prepared as skeletal specimens following thoracic and abdomi-

nal evisceration and subsequent fixation in ethanol for two days for skeletal examinations. The fetuses after fixation were immersed in alizarin red S staining solution (0.0015% alizarin red S, 1.2% potassium hydroxide and 85% ethanol) for 5 days, 1% potassium hydroxide aqueous solution for 2 days and 50% glycerin aqueous solution for 5 days and more. In the skeletal examinations, skeletal abnormalities and mutations were evaluated and the number of the cervical centrums, sternebrae, sacral and coccygeal vertebrae and metacarpals was counted using a stereoscopic microscope (OLYMPUS, Tokyo).

The remaining fetuses were fixed in 10% neutral buffered formalin for at least 1 week and subsequently in Bouin's fluid for at least 2 weeks and were processed for visceral examinations. In the visceral examinations, the presence or absence of visceral abnormalities and mutations was evaluated using the razor blade section method of Wilson (Wilson and Warkany, 1965) for the head and abdomen and by the microdissection method of Nishimura (Nishimura, 1974) for the chest.

These parameters were examined according to the ICH S5 guideline (ICH harmonised tripartite guideline S5 (R2) as revised in 2005, issued in 1993).

Statistical analysis

The mean values and standard deviations for each group were calculated for the body weights, food consumption, the liver and kidney weights, the number of corpora lutea, implantations, live fetuses, dead embryos and dead fetuses, and the total number of dead embryos and fetuses, pre-implantation loss, implantation index, early resorptions, late resorptions, post-implantation loss, incidence of placentae with anomalies, incidence of fetuses with anomalies or variations and number of sacral and coccygeal vertebrae, and the number of metacarpals. Statistical analyses were conducted for comparison of the parameters mentioned above between the control diet group (Group 1) and the fructose containing diet groups (Groups 2 to 5). The levels of significance were set at 5% and 1% (two-tailed) as follows; a Dunnett's test for homogeneous parameters or a Steel's test for non-homogeneous parameters were conducted for comparison of the body weights of dams, organ weights, body weights of live fetuses, placental weights. A Steel's test was conducted for the body weight gain, food consumption, the number of corpora lutea, implantations, live fetuses, dead embryos and dead fetuses, and the total number of dead embryos and fetuses, pre-implantation loss, implantation index, early resorptions, late resorptions, post-implantation loss, incidence of placentae with anomalies, incidence of fetuses with anomalies or variations and the number of the cervical centrums, sternebrae, sacral and coccygeal vertebrae and metacarpals. Fisher's exact test was conducted for the number of dams with placentae having anomalies, the number of dams with fetuses having anomalies or variations, and sex ratio.

RESULTS

Comparison of the dams between the control and fructose diet groups

There were no treatment-related findings in any dam at any diet group in the clinical observations. Body weights were slightly lower in Groups 4 and 5 than those in Group 1 from GDs 3 to 20 (Fig. 1). Food consumption was decreased in Group 5 on GD 3 but turned to increase on GD 13 when compared with Group 1, however total food consumption from GDs 1 to 20 was similar between Groups 1 to 5 (Fig. 2). Mean Fru consumption from GD 1 to 20 was about 3, 7, 10 and 16 g/day in Groups 2 to 5, respectively. At necropsy, enlargement of the liver was observed in Groups 2 to 5 and pale discoloration of the liver was observed in Groups 3 to 5. The liver and kidney weights were higher in Groups 3 to 5 than those in Group 1 (Table 2).

Comparison of the fetuses between the control and fructose diet groups

There were no effects of the fructose diets on the data from the caesarean section including the number of corpora lutea and implantations, the implantation index, the pre-implantation loss, post-implantation loss, the body weights of live fetuses, the placental weights or the sex ratio in any of the fructose diet group (Table 3). There were no treatment-related external findings in the live fetuses in any of the fructose diet groups (Table 4).

The data for skeletal examinations are shown in Table 5. There were no treatment-related skeletal anomalies or variations in any of the fructose diet groups. Detached ribs were observed in Group 2. The finding was considered to be incidental because these were limited to one fetus and there were no differences in the incidences between the control and any of the fructose diet groups. Short 13th rib, short supernumerary rib, incomplete ossification of cervical arch, incomplete ossification of hyoid, incomplete ossification of the sternebrae, bipartite ossification of the thoracic centrum, dumbbell ossification of the thoracic centrum and asymmetric sternebrae were observed in Groups 2 to 5. However, there were no clear differences in the incidence between the control and any of the fructose diet groups. There were no clear differences in the number of the cervical centrums, sternebrae,

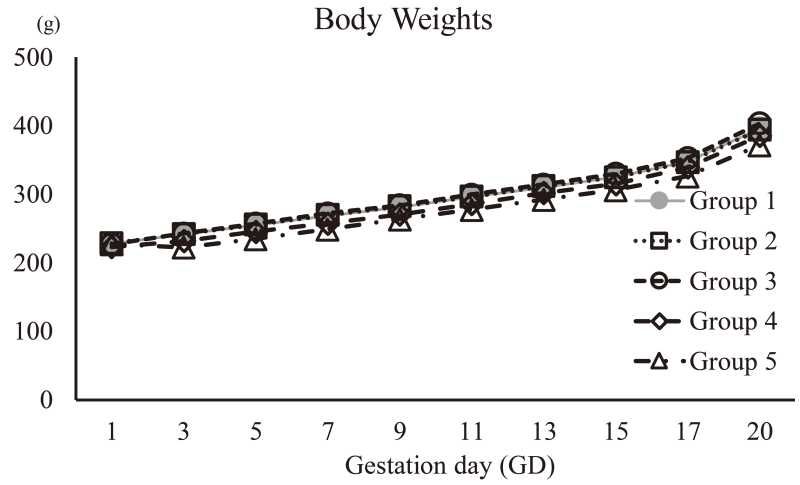

Fig. 1. The body weights (g) during pregnancy. Fructose diet was fed to the animals from GDs 1 to the day of caesarean section (GD20). The body weights were slightly lower in Groups 4 and 5 than those in Group 1 from GDs 3 to 20 but there were no significant differences.

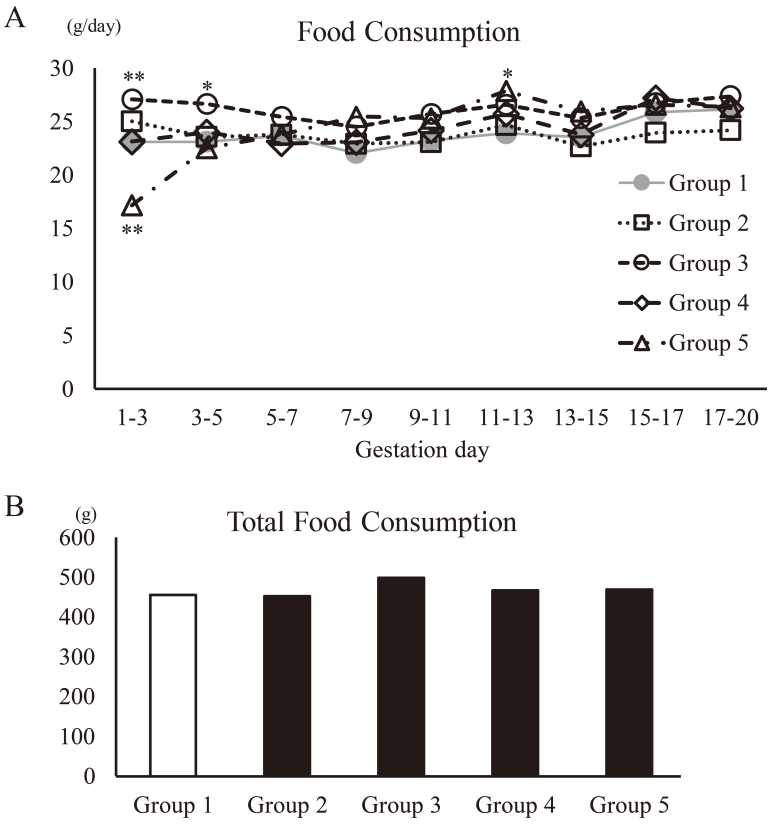

Fig. 2. A: The food consumption (g/day) during pregnancy. The food consumption was decreased in Group 5 on GD 3 ($p < 0.01$) and turned to increase on GD 13 ($p < 0.05$) when compared with Group 1. * $p < 0.05$, ** $p < 0.01$ (Steel's test), B: Total food consumption (g) from GD 1 to 20. There were no significant differences.

Table 1. Comparison of the control and high fructose diets.

	Control Diet	High Fructose Diets			
Group Number	Group 1	Group 2	Group 3	Group 4	Group 5
Diet Name	D11708C (g/100 g)	D18071207 (g/100 g)	D18071206 (g/100 g)	D18071205 (g/100 g)	D11707R (g/100 g)
Protein	20	20	20	20	20
Fat	5	5	5	5	5
Mineral	4	4	4	4	4
Fiber	5	5	5	5	5
Carbohydrate	66	66	66	66	66
Fructose content in Carbohydrate	0	13	26	39	65
Calorie (kcal/ 100 g)	390	390	390	390	390

Table 2. The summary of organ weights and necropsy of the liver and kidney.

	Control Diet	High Fructose Diets			
	Group 1	Group 2	Group 3	Group 4	Group 5
Fructose content (g/100 g)	0	13	26	39	65
Number of dams	10	10	10	8†	10
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Final body weights (g)	395.8 ± 23.8	394.2 ± 39.4	403.6 ± 32.7	365.8 ± 47.4	371.6 ± 40.1
Absolute liver weight (g)	13.9 ± 1.4	14.6 ± 1.7	16.2 ± 1.5	14.4 ± 2.9	16.2 ± 2.6
Relative liver weight (g/100gBW)	4.3 ± 0.3	4.6 ± 0.2	5.0 ± 0.4**	4.7 ± 0.8	5.5 ± 0.6**
Absolute kidney weight (mg)	1907 ± 201	1915 ± 212	2161 ± 276	1959 ± 166	2075 ± 306
Relative kidney weight (mg/100gBW)	590 ± 33	606 ± 32	663 ± 65**	635 ± 33	698 ± 51**
Macroscopic findings‡					
Liver					
Large	-	+: 1	+: 1	+: 2	+: 3
Discoloration pale	-	-	P: 2	P: 2	P: 3

**: Statistically significant between the control diet group (Group 1) and high fructose diet groups (Group 2 to 5). ** $p < 0.01$ (Dunnett's test)†: Two animals in the group 4 were non-pregnant. ‡: Criteria for grading macroscopic findings: -: No abnormal changes, + : Slight, 2+ : Moderate, 3+ : Severe, P : Finding presence (non-graded finding). Number of animals in which the grade was observed.

sacral and coccygeal vertebrae and metacarpals between the control and any of the fructose diet groups.

The data for the visceral examinations are shown in Table 6. There were no treatment-related visceral anomalies or variations in any fructose diet group. Thymic remnant in the neck, persistent left umbilical artery, dilated renal pelvis and dilated ureters were observed in Groups 2 to 5. However, there were no clear differences in the incidence of any of above findings between the control and any fructose diet group.

DISCUSSION

In the present study, pregnant rats were fed a control diet or high fructose diets (containing 13%, 26%, 39% and 65% fructose) from GDs 1 to 20 and the effects of a high fructose diet on the dams and fetuses were examined by mesurements and examinations according to ICH S5 guideline.

A decrease in the food consumption was noted in the high fructose diets group containing 65% fructose up to GD 3 but turned into an increase on GD 13. There were no differences in the total food consumption between the control and any of the fructose diet groups and it was considered that the fructose intake from the diet increased concentration-dependently. The Fru intake of each Groups 2 to 5 was equivalent to about 500, 1000, 1500 and 2500 g/man/day in humans (60 kg). The intakes at even the lowest dose level were ten-times above those at which effects have been reported in humans (49 g/ day).

Increased liver weights, and pale discoloration and enlargement of the liver were observed Fru concentration-dependently in their incident/severity in all the fructose diet groups. It has been reported that the dietary fructose intake during pregnancy in rats resulted in the development of gestational diabetes coupled with hepatic ste-

Table 3. The summary of caesarean section data.

	Control Diet	High Fructose Diets			
	Group 1	Group 2	Group 3	Group 4	Group 5
Fructose content (g/100 g)	0	13	26	39	65
Number of dams	10	10	10	8†	10
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Number of corpora lutea	14.6 ± 1.9	15.1 ± 2.1	15.1 ± 1.7	14.5 ± 1.2	14.7 ± 1.7
Number of implantations	14.0 ± 1.3	14.8 ± 1.9	14.7 ± 1.8	13.8 ± 1.2	14.1 ± 1.6
Implantation index (%)	96.5 ± 7.1	98.2 ± 4.1	97.3 ± 3.6	94.9 ± 4.8	96.1 ± 4.8
Pre-implantation loss (%)	3.5 ± 7.1	1.8 ± 4.1	2.7 ± 3.6	5.1 ± 4.8	4.0 ± 4.8
Number of dead embryos and fetuses					
Early	0.5 ± 0.8	0.4 ± 0.7	0.3 ± 0.7	1.0 ± 1.1	0.5 ± 0.7
Late	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.4	0.0 ± 0.0
Total	0.5 ± 0.8	0.4 ± 0.7	0.3 ± 0.7	1.1 ± 1.0	0.5 ± 0.7
Post-implantation loss (%)					
Early	3.6 ± 6.0	2.6 ± 4.8	1.8 ± 4.2	7.1 ± 7.5	3.5 ± 5.0
Late	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.8 ± 2.4	0.0 ± 0.0
Total	3.6 ± 6.0	2.6 ± 4.8	1.8 ± 4.2	7.9 ± 7.0	3.5 ± 5.0
Number of live fetuses					
Male	7.3 ± 1.5	7.7 ± 2.5	6.9 ± 3.1	6.4 ± 2.0	7.0 ± 1.9
Female	6.2 ± 1.8	6.7 ± 1.6	7.5 ± 2.0	6.3 ± 2.1	6.6 ± 1.6
Total	13.5 ± 1.6	14.4 ± 1.8	14.4 ± 1.6	12.6 ± 1.1	13.6 ± 1.6
Sex ratio (%, Male/Total)	54.1	53.5	47.9	50.5	51.5
Body weights of live fetuses					
Male	4.00 ± 0.28	3.92 ± 0.25	3.97 ± 0.43	3.99 ± 0.18	3.97 ± 0.34
Female	3.78 ± 0.27	3.69 ± 0.18	3.82 ± 0.45	3.90 ± 0.18	3.80 ± 0.15
Placental weights of live fetuses					
Male	0.41 ± 0.04	0.41 ± 0.05	0.41 ± 0.07	0.45 ± 0.04	0.42 ± 0.05
Female	0.40 ± 0.04	0.42 ± 0.05	0.41 ± 0.07	0.43 ± 0.05	0.40 ± 0.04

†: Two animals in the group 4 were non-pregnant.

Table 4. The summary of external examination.

	Control Diet	High Fructose Diets			
	Group 1	Group 2	Group 3	Group 4	Group 5
Fructose content (g/100 g)	0	13	26	39	65
Number of dams	10	10	10	8†	10
Number of fetuses	135	144	144	101	136
Number of Fetuses with any Anomalies	0	1	0	0	0
Mean % of Fetuses with Anomaly	0.00	0.83	0.00	0.00	0.00
Ectrodactyly					
Number of Fetuses	0	1	0	0	0
Mean % of Fetuses with Anomaly	0.00	0.83	0.00	0.00	0.00
Dwarf					
Number of Fetuses	0	1	0	0	0
Mean % of Fetuses with Anomaly	0.00	0.83	0.00	0.00	0.00

†: Two animals in the group 4 were non-pregnant.

Table 5. The summary of skeletal examination.

	Control Diet	High Fructose Diets			
	Group 1	Group 2	Group 3	Group 4	Group 5
Fructose content (g/100 g)	0	13	26	39	65
Number of dams	10	10	10	8†	10
Number of fetuses	65	69	70	49	65
Anomalies					
Number of Fetuses with any Anomalies	1	1	0	0	0
Mean % of Fetuses with Anomaly	1.43	1.67	0.00	0.00	0.00
Detached rib					
Number of Fetuses	0	1	0	0	0
Mean % of Fetuses	0.00	1.67	0.00	0.00	0.00
Fused sternebra					
Number of Fetuses	1	0	0	0	0
Mean % of Fetuses	1.43	0.00	0.00	0.00	0.00
Variations					
Number of Fetuses with any Variations	5	6	9	4	5
Mean % of Fetuses with Variation	7.68	8.52	12.54	7.75	7.62
Short 13th rib					
Number of Fetuses	3	0	0	1	0
Mean % of Fetuses	4.77	0.00	0.00	2.09	0.00
Short supernumerary rib					
Number of Fetuses	0	2	2	3	2
Mean % of Fetuses	0.00	2.92	3.43	5.66	3.10
Incomplete ossification of cervical arch					
Number of Fetuses	0	1	0	0	0
Mean % of Fetuses	0.00	1.67	0.00	0.00	0.00
Incomplete ossification of hyoid					
Number of Fetuses	1	0	1	0	1
Mean % of Fetuses	1.25	0.00	1.43	0.00	1.67
Incomplete ossification of sternebra					
Number of Fetuses	0	1	0	0	0
Mean % of Fetuses	0.00	1.67	0.00	0.00	0.00
Bipartite ossification of thoracic centrum					
Number of Fetuses	1	1	0	0	1
Mean % of Fetuses	1.67	1.67	0.00	0.00	1.43
Dumbbell ossification of thoracic centrum					
Number of Fetuses	0	3	5	0	1
Mean % of Fetuses	0.00	3.93	6.43	0.00	1.43
Asymmetric sternebra					
Number of Fetuses	0	0	1	0	0
Mean % of Fetuses	0.00	0.00	1.25	0.00	0.00
Number of Ossification (Mean ± SD)					
Cervical centrum	0.95 ± 0.76	0.92 ± 0.62	1.25 ± 1.56	0.89 ± 0.93	0.74 ± 0.59
Sternebra	5.76 ± 0.32	5.83 ± 0.25	5.70 ± 0.36	5.90 ± 0.14	5.83 ± 0.17
Sacral and coccygeal vertebra	8.48 ± 0.39	8.43 ± 0.34	8.18 ± 0.75	8.33 ± 0.30	8.63 ± 0.32
Metacarpus	7.91 ± 0.17	7.64 ± 0.42	7.75 ± 0.30	7.85 ± 0.25	7.81 ± 0.57

†: Two animals in the group 4 were non-pregnant.

Table 6. The summary of visceral examination.

	Control Diet	High Fructose Diets			
	Group 1	Group 2	Group 3	Group 4	Group 5
Fructose content (g/100 g)	0	13	26	39	65
Number of dams	10	10	10	8†	10
Number of fetuses	65	69	70	49	65
Anomalies					
Number of Fetuses with any Anomalies	0	0	0	0	0
Mean % of Fetuses with Anomaly	0.00	0.00	0.00	0.00	0.00
Variations					
Number of Fetuses with any Variations	9	8	13	9	11
Mean % of Fetuses with Variation	12.99	10.46	17.21	16.69	15.13
Thymic remnant in neck					
Number of Fetuses	3	2	4	2	3
Mean % of Fetuses	4.35	2.54	5.60	3.58	4.17
Persistent left umbilical artery					
Number of Fetuses	1	0	1	0	1
Mean % of Fetuses	1.25	0.00	1.25	0.00	1.43
Dilated renal pelvis					
Number of Fetuses	1	0	1	0	1
Mean % of Fetuses	1.43	0.00	1.25	0.00	1.25
Dilated ureter					
Number of Fetuses	6	7	9	8	9
Mean % of Fetuses	8.82	9.35	11.79	14.90	12.45

†: Two animals in the group 4 were non-pregnant.

atosis (Zou *et al.*, 2012). Fru increases glycerol 3-phosphate, which is used as a metabolite in the production of triglycerides in the liver (Federico *et al.*, 2021; Herman and Samuel, 2016). Chronic ingestion also activates the major transcription factors of *de novo* lipogenesis, including sterol regulatory element-binding protein 1c (SREBP1c) and carbohydrate-reactive protein 1c (ChREBP). SREBP1c and ChREBP potently induce lipogenesis by triggering the activation of genes for fatty acid synthase and acetyl-coA carboxylase (Federico *et al.*, 2021; Geidl-Flueck and Gerber, 2017). Therefore, the above findings noted in the present study were considered to be similar to those profiles and to be related to fructose-induced lipidosis in the liver.

The kidney weights were increased in the 26% fructose diet group and above. In the microscopic examination, tubular lesions including hyaline cast, epithelial regeneration, dilatation and mineralization in the kidney were observed in the dam in the 65% fructose diet group. It has been reported that a decrease in glomerular filtration rate and an increase in blood pressure were observed in pregnant and lactating female rats given 20% fructose aqueous solution ad libitum (Monteiro *et al.*, 2022). In addition, the intake of 63% fructose diet exaggerated the renal lesions in the non-obese type 2 diabetes model, Jcl:SDT (SDT) rats, which spontaneously develop hyperglycemia and glucose intolerance (Toyoda *et al.*, 2018). Renal findings noted in this study are very similar to the findings reported in SDT rats. Therefore, it is likely that the kidney findings noted in the present study were similar to those in SDT rats because of physiological hyperglycemia during pregnancy in rats (Herrera *et al.*, 1988).

In the comparison between the control diet group and fructose diet groups in the fetuses, there were no fructose-related findings/changes in any high fructose diet group for the number of corpora lutea, the number of implantations, the implantation index, the pre-implantation loss, post implantation loss, the body weights of live fetuses, placental weights, the sex ratio, the incidence of external, skeletal and visceral anomalies/variations or the number of the cervical centrums, sternebrae, sacral and coccygeal vertebrae and metacarpals. These results revealed that the high fructose intake during pregnancy in the rats does not cause fetal morphological changes. However, it has been reported that oral administration of fructose (about 850 g/man/day in humans) to dams from GDs 1 to 10 causes low plasma insulin levels in the offspring (Vickers *et al.*, 2011) and feeding dams with high-fructose water (about 3600 g/man/day in human) from 12 weeks before mating until weaning causes neurodevel-

opmental deficits (effects of the behavioral tests, gliosis and neuronal cell death) in the offspring in rats (Erbas *et al.*, 2018). However, there were no fetal findings associated with these changes reported in offspring in the present study. Detection of the fructose-related findings in the offspring reported in the literature were considered to be technically unadaptable with the examinations that are usually performed in embryo-fetal development studies followed by ICH S5 guideline and it is unclear whether the fructose-related findings in the offspring became obvious from the fetal period or after birth.

In conclusion, dietary high fructose intake during pregnancy in the rats caused fatty change in the liver suggestive of fatty liver and effects on the kidney in the dams but did not cause fetal morphological changes, even at doses exceeding the human health-effect level by more than 50-fold.

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Conflict of interest---- The authors declare that there is no conflict of interest.

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