Original Article

Maternal high-fructose consumption provokes placental oxidative stress resulting in asymmetrical fetal growth restriction in rats

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We aimed to determine the impact of high-fructose intake during pregnancy on the fetal-placental unit in rats, which may be the initial mechanism of the programming effect of fructose. Pregnant Sprague–Dawley rats were randomly assigned to three groups and respectively provided tap water (n = 10), 10% (w/v) fructose solution (n = 10), and 10% (w/v) glucose solution (n = 10) from embryonic day 0 to 20. Compared with the control and glucose groups, significantly lower fetal length, fetal weight, placental weight, and fetus/placenta ratio were found in the fructose group on embryonic day 20 (all p<0.05). In parallel with markedly increased uric acid concentrations in the dams, significantly decreased antioxidant enzymes activities and mRNA expression levels were observed in placentas in the fructose group (all p<0.05). In the fructose group, placental mRNA and protein expression of nuclear factor erythroid 2-related factor 2 was markedly downregulated and kelch-like ECH-associated protein 1 was significantly upregulated (all p<0.05). In conclusion, high-fructose consumption during pregnancy drives augmented oxidative stress in rats. Placental insufficiency under oxidative stress contributes to asymmetrical fetal growth restriction.

Key Words: **fructose, placenta, uric acid, oxidative stress, fetal growth restriction**

In recent decades, dietary fructose intake has increased significantly, driven by its wide use as a sweetener in processed foods and beverages. Fructose is an isomer of glucose, and has the same molecular formula as glucose but a different structure. Glucose is tightly regulated to produce ATP. However, fructose has a special metabolic pathway which is different to that of glucose: ingested fructose is extracted in the liver, where it is rapidly phosphorylated by fructokinase and converts to fructose-1-phosphate with depletion of ATP and activation of AMP deaminase. AMP is then converted to xanthine, which is converted to uric acid (UA) by xanthine oxidase (XOD).[1]

Increasing experimental and epidemiological studies have demonstrated that maternal diet manipulation during pregnancy can markedly affect the physiology and metabolism of the offspring, which is called nutritional programming.[2,3] Pregnant women are exposed to the same artificially sweetened foods and beverages as the general population. It has been found that maternal fructose consumption can increase the risk of gallbladder disease and gestational diabetes mellitus and is associated with pre-eclampsia and preterm delivery.[4–7] Furthermore, maternal fructose consumption during pregnancy and lactation can lead to insulin resistance,[8] fatty liver,[9] adipose tissue dysfunction,[10] hypoadiponectinemia,[11] dyslipidemia, and endocrine function changes in the offspring.[12,13] However, the mechanism has not been completely defined.

During pregnancy, the placenta constitutes the active interface between the maternal and fetal blood circulations, mediating the transfer of nutrients and regulating fetal growth.[14] Perturbations in the maternal environment must be transmitted across the placenta in order to affect the fetus. The placenta plays a crucial role in protecting the fetus from adverse effects. Therefore, placental change may be the initial factor in nutritional programming. However, the effect of maternal fructose consumption on the placenta has not received enough attention.[13]

The placenta is prone to attack by oxidants present in the maternal circulation. Under physiological circumstances, antioxidant enzyme and non-enzymatic scavengers in the placenta can defend against reactive oxygen species (ROS) and ensure normal fetal growth.[15] However, when oxidative insult is present for a long period, the placenta may undergo oxidative damage, lipoperoxidation products are then released into the circulation, subsequently causing endothelial cell impairment and ultimately affecting fetal growth and development.[16] Oxidative stress in the placenta has been found to cause placental dysfunction and was associated with early pregnancy failure and fetal malformations.[15] In addition, placental oxidative stress is even considered to be one of the possible mechanisms underlying nutritional programming.[17]

Fructose can participate in glycosylation reactions which generate free radicals and alter homeostasis redox.[18] Additionally, fructose induces *de novo* UA synthesis, which can aggravate oxidative stress.[19,20] Accordingly, we performed the present animal study to investigate the effect of maternal high-fructose consumption during pregnancy on placental oxidative stress and fetal growth.

Material and Methods

Animals and experimental design. Female Sprague–Dawley rats weighing 220 ± 20 g was sourced from Shandong Lukang Laboratory Animal Center (Qingdao, China). The female rats were mated overnight with male rats. Successful mating was defined as the day on which the presence of a sperm-positive vaginal smear was found and was designated embryonic day 0 (ED0). Matched for body weight, the pregnant rats were randomly assigned to the control group (CON, n = 10), fructose group (FRU, n = 10), and glucose group (GLU, n = 10). The FRU and GLU groups received a 10% (w/v) solution in drinking water throughout their pregnancy. The CON group was provided tap water with no supplementary sugar. All pregnant rats were

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Table 1. Primer Sequences for quantitative real-time PCR

Gene		Sequence	Product length (bp)
SOD	Forward	5'-ACCTCAATCGCCTCTGTGC-3'	201
	Reverse	5'-GGAGTTGGTCTGTGGAGTGC-3'	
CAT	Forward	5'-ACATAGCTGCCAAGGGAAAA-3'	108
	Reverse	5'-GATTACTGGTGAGGCTTGTGC-3'	
GSH-Px	Forward	5'-TGCTGGCAAATACATCCTCTT-3'	248
	Reverse	5'-CGTTCACGTCTCCTTTCTCAA-3'	
HO-1	Forward	5'-GAATCGAGCAGAACCAGCCT-3'	135
	Reverse	5'-CTCAGCATTCTCGGCTTGGA-3'	
NQO1	Forward	5'-CATCATTTGGGCAAGTCC-3'	197
	Reverse	5'-ACAGCCGTGGCAGAAC-3'	
Nrf2	Forward	5'-GAATAAAGTTGCCGCTCAGAA-3'	209
	Reverse	5'-AAGGTTTCCCATCCTCATCAC-3'	
Keap1	Forward	5'-GAAGAGGCAGCAGAACAAGC-3'	201
	Reverse	5'-GGGTGTGGGTGGTAGGAGTT-3'	
β-actin	Forward	5'-AGGGAAATCGTGCGTGAC-3'	146
	Reverse	5'-CGCTCATTGCCGATAGTG-3'	

CAT, catalase; GSH-Px, glutathione peroxidase; HO-1, heme oxygenase-1; Keap1, kelchlike ECH-associated protein 1; MDA, malonaldehyde; NQO1, NAD(P)H quinone oxidoreductase 1; Nrf2, nuclear factor erythroid 2-related factor 2; SOD, superoxide dismutase.

fed a standard rodent chow (AIN-93G, Keaoxieli Fodder, Beijing, China) and housed individually. Food and fluid were freely available, and their intake was recorded daily. Body weights were measured weekly.

On ED20, the dams were weighed, fasted overnight and anesthetized. Blood was collected, placentas and fetuses were removed, counted and weighed. The fetal weight to placental weight ratio was calculated. The head to tail distance of the fetuses were measured using a digital caliper. Fetal blood samples were collected by decapitation. The liver and brain from each fetus were removed and weighed. Fetal liver and brain to body weight ratios were calculated, respectively. Placentas taken from the same litter were pooled. Blood samples from all fetuses from the same dam were pooled. Blood and tissues samples were stored at −80°C.

All experimental procedures and protocols followed the guidelines for the care and use of animals, which were established at Medical College of Qingdao University and approved by the Animal Experimentation Ethics Committee of Medical College of Qingdao University.

Serum measurements. Fasting serum glucose (FG), triglyceride (TG), and UA were measured by an automatic biochemistry analyzer (PM4000; Autolab, Rome, Italy). ELISA kits were used to determine the concentrations of fasting insulin (FIN) (EZRMI-13K; Merck Millipore, Billerica, MA), fructose, malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), placental growth factor (PLGF), and soluble fms-like tyrosine kinase-1 (sFlt-1) (all from Jiancheng Technology, Nanjing, China). Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as [FG (mmol/L) × FIN (mIU/L)]/22.5.

Placental tissue measurements. Placental tissue was homogenized and centrifuged at 4°C. The supernatant was used to measure the concentrations of UA and MDA using ELISA kits (all from Jiancheng Technology). The activities of XOD, SOD, CAT, and GSH-Px in the placenta were examined using commercial kits (all from Jiancheng Technology) according to the manufacturer's instructions.

RNA isolation Quantitative real-time PCR. Total RNA was extracted from placenta with TRIzol Reagent (Invitrogen, Karlsruhe, Germany) according to the manufacturer's protocol. cDNA was synthesized with the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA). The specific products were amplified and detected with the Applied Biosystems 7500 Fast Real-Time PCR System (Applied Biosystems). The primer sequences used are shown in Table 1.

Western blotting. The protocol for western blotting has been described previously.[21] The antibodies used were as follows: nuclear factor erythroid 2-related factor 2 (Nrf2), kelch-like ECH-associated protein 1 (Keap1), and Lamin B1 (Abcam, Cambridge, MA); β-actin and secondary antibody (Santa Cruz Biotechnology, Santa Cruz, CA).

Statistical analysis. Statistical analyses were performed with IBM SPSS Statistics ver. 22.0. The results are presented as means ± SD. One-way analysis of variance was used to assess the differences between the groups. Statistical significance was set at $p<0.05$.

Results

Maternal energy intake and body weight during pregnancy. Compared with the CON group, the pregnant rats in the FRU group showed a marked decrease in daily food intake and a significant increase in daily fluid intake. However, in comparison to the GLU group, pregnant rats in the FRU group had markedly higher daily food intake and significantly lower daily fluid intake. The mean daily total energy intake during pregnancy was not significantly different between the three groups. In parallel with the energy intake, no significant difference was found in body weight between the three groups on ED20. Neither fructose nor glucose consumption altered the increase in maternal body weight during pregnancy (Table 2).

Placental and fetal characteristics. No significant difference was found in litter size, liver/fetal weight and brain/fetal weight among the three groups. Compared with the CON and GLU groups, the FRU group had significantly lower fetal length, fetal weight, placental weight and fetus/placenta ratio (Table 3).

Maternal and fetal serum fructose concentrations. Maternal (Fig. 1A) and fetal (Fig. 1B) serum fructose concentrations in the FRU group were significantly higher than those in the

Table 2. Maternal energy intake and body weight during pregnancy

	CON	FRU	GLU
Food intake (g/day)	24.39 ± 1.86[a]	17.90 ± 1.63[b]	14.68 ± 2.09[c]
Fluid intake (ml/day)	37.77 ± 3.37[a]	69.05 ± 15.01[b]	110.67 ± 22.33[c]
Total energy intake (kcal/day)	96.83 ± 7.39	101.43 ± 10.27	106.98 ± 15.11
Body weight (g)			
On ED0	241.33 ± 15.69	244.46 ± 12.06	247.50 ± 15.09
On ED20	354.77 ± 21.57	359.11 ± 27.96	372.51 ± 39.27

Data are expressed as mean ± SD. Values in the same row with different superscripts are significantly different from each other (p<0.05). CON, control; ED, embryonic day; FRU, fructose; GLU, glucose.

Table 3. Placental and fetal characteristics

	CON	FRU	GLU
Litter size (n)	14.00 ± 1.05	13.80 ± 0.92	15.00 ± 1.06
Placental weight (g)	0.74 ± 0.08[a]	0.53 ± 0.12[b]	0.71 ± 0.14[a]
Fetal length (mm)	23.60 ± 1.51[a]	18.89 ± 1.75[b]	22.22 ± 1.36[a]
Fetal weight (g)	6.73 ± 0.61[a]	3.61 ± 0.71[b]	6.07 ± 0.92[a]
Fetus/placenta ratio	9.14 ± 0.55[a]	6.82 ± 0.42[b]	8.68 ± 0.58[a]
Liver/fetal weight (%)	4.31 ± 0.39	4.12 ± 0.44	4.20 ± 0.31
Brain/fetal weight (%)	4.54 ± 0.30	4.32 ± 0.37	4.43 ± 0.35

Data are expressed as mean ± SD. Values in the same row with different superscripts are significantly different from each other (p<0.05). CON, control; FRU, fructose; GLU, glucose.

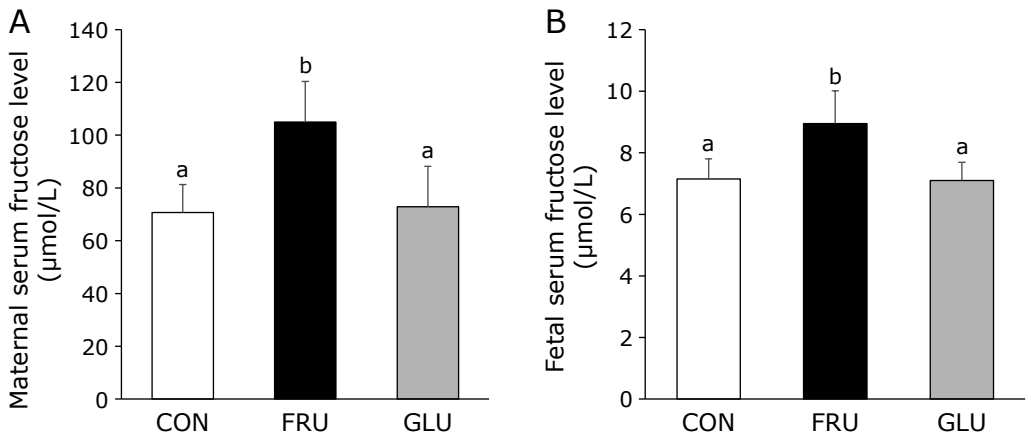

Fig. 1. Maternal (A) and fetal (B) serum fructose concentrations. Values are means ± SD. Values with different letters are significantly different from each other (p<0.05); n = 10 per group. CON, control; FRU, fructose; GLU, glucose.

CON and GLU groups. No differences in maternal and fetal serum fructose concentrations between the CON and GLU groups were found.

Maternal and fetal serum glucose and lipid metabolism indices. There were no significant differences in maternal and fetal serum FG, FIN, or HOMA-IR levels among the three groups (Table 4). Compared with the CON and GLU groups, the FRU group had markedly elevated maternal and fetal serum TG and UA concentrations. Furthermore, significantly higher TG concentrations were found in the maternal serum of the GLU group compared with the CON group.

Maternal and fetal serum oxidative stress parameters. The FRU group had markedly elevated MDA and reduced CAT, SOD, and GSH-Px levels in maternal and fetal serum compared with the CON and GLU group (Table 5). In addition, compared with the CON group, significantly decreased MDA and increased CAT, SOD, and GSH-Px levels were found in the maternal and fetal serum of the GLU group.

Maternal serum PLGF and sFlt-1 concentrations. Compared with the CON and GLU groups, the FRU group had significantly decreased maternal serum PLGF concentrations and markedly increased sFlt-1 concentrations (Fig. 2A and B). The ratio of sFlt-1/PLGF was significantly higher in the FRU group than that in the CON and GLU groups (Fig. 2C).

Placental XOD activity and UA concentration. The placental XOD activity and UA concentration in the FRU group were significantly higher than those in the CON group (Fig. 3). However, compared with the GLU group, the placental XOD activity tended to increase and the UA concentration was significantly elevated in the FRU group. The GLU group had a markedly higher UA concentration compared with the CON group.

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Table 4. Maternal and fetal serum glucose and lipids metabolism indices levels.

	Maternal			Fetal		
	CON	FRU	GLU	CON	FRU	GLU
FG (mmol/L)	5.17 ± 0.38	4.94 ± 0.18	4.98 ± 0.17	2.68 ± 0.38	3.31 ± 0.90	3.13 ± 0.92
FIN (pmol/L)	34.56 ± 6.56	37.65 ± 6.31	33.52 ± 4.50	61.82 ± 17.29	57.58 ± 23.35	59.37 ± 20.24
HOMA-IR	1.13 ± 0.17	1.18 ± 0.17	1.07 ± 0.14	1.04 ± 0.24	1.11 ± 0.22	1.11 ± 0.24
TG (mmol/L)	0.67 ± 0.25[a]	1.75 ± 0.27[b]	0.98 ± 0.30[c]	0.46 ± 0.03[a]	0.63 ± 0.07[b]	0.50 ± 0.04[a]
UA (μg/ml)	328.50 ± 30.04[a]	949.10 ± 114.62[b]	396.26 ± 61.27[a]	61.67 ± 12.90[a]	90.37 ± 24.04[b]	73.45 ± 13.38[a]

Data are expressed as mean ± SD. Data of dams and fetuses were separately analyzed. Values in the same row with different superscripts are significantly different from each other (p<0.05). CON, control; FG, fasting glucose; FIN, fasting insulin; FRU, fructose; GLU, glucose; HOMA-IR, homeostasis model assessment of insulin resistance; TG, triacylglycerol; UA, uric acid.

Table 5. Maternal and fetal serum oxidative stress parameters levels

	Maternal			Fetal		
	CON	FRU	GLU	CON	FRU	GLU
MDA (nmol/ml)	3.84 ± 0.32[a]	6.91 ± 0.64[b]	4.99 ± 0.78[c]	1.91 ± 0.27[a]	3.94 ± 0.33[b]	2.46 ± 0.37[c]
CAT (U/ml)	82.72 ± 7.90[a]	47.10 ± 5.31[b]	60.80 ± 8.04[c]	36.24 ± 4.57[a]	20.30 ± 2.47[b]	27.11 ± 3.91[c]
SOD (U/ml)	10.31 ± 0.52[a]	5.52 ± 0.75[b]	6.68 ± 1.15[c]	7.23 ± 1.40[a]	3.76 ± 0.44[b]	4.98 ± 0.40[c]
GSH-Px (U/ml)	29.05 ± 1.38[a]	19.16 ± 1.21[b]	24.00 ± 0.72[c]	20.33 ± 1.93[a]	12.56 ± 2.87[b]	16.01 ± 3.91[c]

Data are expressed as mean ± SD. Data of dams and fetuses were separately analyzed. Values in the same row with different superscripts are significantly different from each other (p<0.05). CAT, catalase; CON, control; FRU, fructose; GLU, glucose; GSH-Px, glutathione peroxidase; MDA, malonaldehyde; SOD, superoxide dismutase.

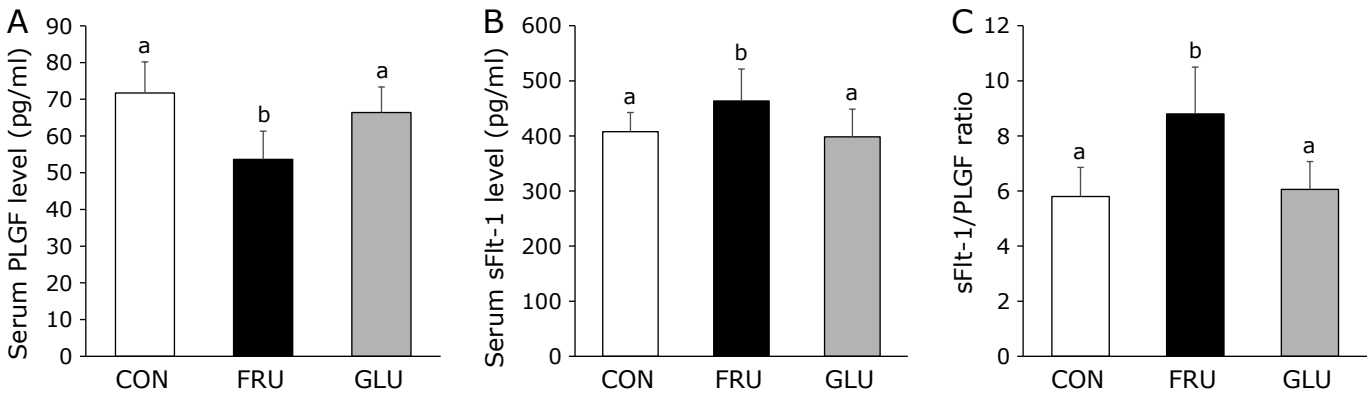

Fig. 2. Maternal serum PLGF (A), sFlt-1 (B), and sFlt-1/PLGF ratio (C) levels. Values are means ± SD. Values with different letters are significantly different from each other (p<0.05); n = 10 per group. CON, control; FRU, fructose; GLU, glucose; PLGF, placental growth factor; sFlt-1, soluble fms-like tyrosine kinase-1.

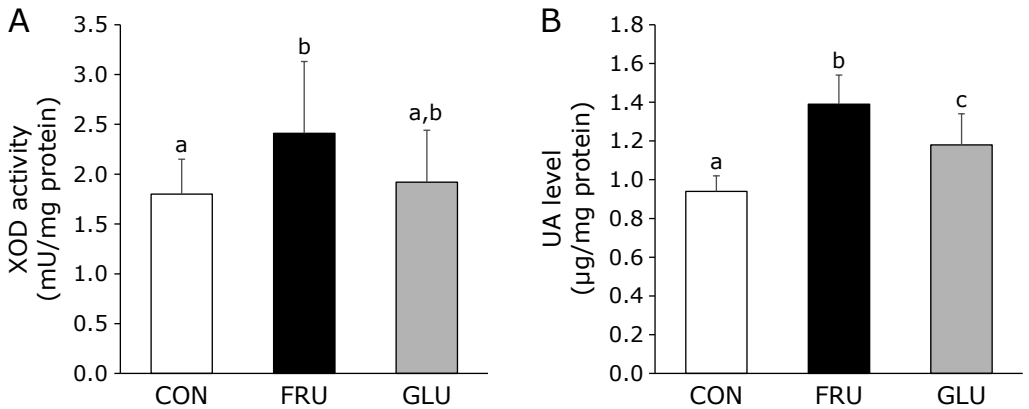

Fig. 3. Placental XOD activity (A) and UA concentration (B). Values are means ± SD. Values with different letters are significantly different from each other (p<0.05); n = 10 per group. CON, control; FRU, fructose; GLU, glucose; UA, uric acid; XOD, xanthine oxidase.

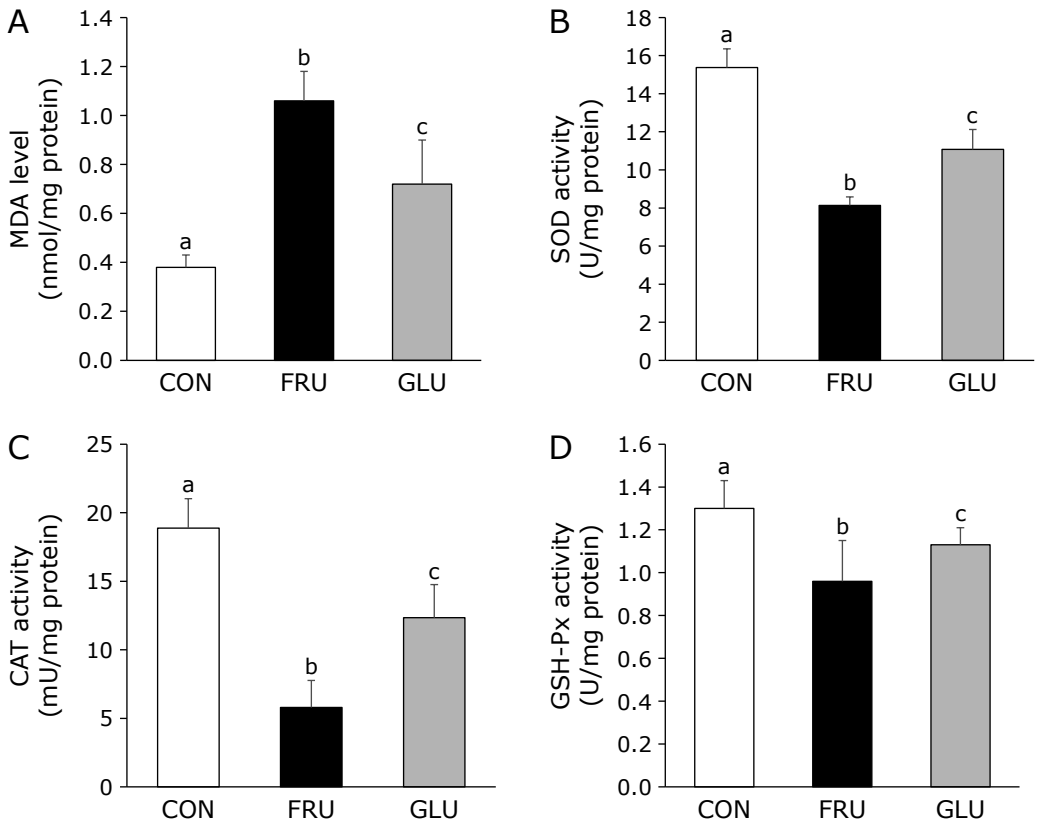

Fig. 4. Placental oxidative stress parameters levels. Values are means ± SD. Values with different letters are significantly different from each other (p<0.05); n = 10 per group. CAT, catalase; CON, control; FRU, fructose; GLU, glucose; GSH-Px, glutathione peroxidase; MDA, malonaldehyde; SOD, superoxide dismutase.

Placental oxidative stress parameters measurement. The placental MDA concentration was significantly higher and the placental SOD, CAT, and GSH-Px activities were markedly lower in the FRU group than those in the CON and GLU groups (Fig. 4). Additionally, in comparison to the CON group, the MDA concentration was significantly increased and the SOD, CAT, and GSH-Px activities were all markedly decreased in placentas in the GLU group.

mRNA expression levels of placental oxidative stress parameters. The mRNA expression of SOD, CAT, GSH-Px, heme oxygenase-1 (HO-1), and NAD(P)H quinone oxidoreductase 1 (NQO1) in the placenta were significantly lower in the FRU group than in the CON and GLU groups (Fig. 5). Moreover, the GLU group had markedly decreased SOD, CAT, GSH-Px, HO-1, and NQO1 mRNA expression levels in the placenta in comparison to the CON group.

Placental Nrf2 and Keap1 mRNA and protein expression levels. Compared with the CON and GLU groups, the FRU group had significantly decreased placental Nrf2 and increased Keap1 mRNA and protein expression levels (Fig. 6A, B, D, and F). Furthermore, the GLU group had significantly down-regulated placental Nrf2 and up-regulated Keap1 mRNA and protein expression levels compared with the CON group.

Discussion

The results of the present study suggest that maternal high-fructose intake during pregnancy induced placental oxidative stress. Oxidative impairment of the placenta ultimately contributed to asymmetrical fetal growth restriction (AFGR) in rats.

In recent decades, free fructose consumption has increased in all gender and age groups with the introduction of both fructose and high fructose syrup as sweeteners, which are widely used in processed foods and beverages. Currently, more attention is paid to excess fructose consumption as a few adverse influences have been found, including its negative impact on nutritional programming. In early studies, it was shown that a maternal 50–60% fructose diet or 20% fructose solution resulted in elevations in serum TG, glucose, and insulin concentrations in the offspring;[12,13] however, fructose intake in these studies far exceeded that of normal human intake. Therefore, in the present study, we provided pregnant rats with 10% (w/v) fructose solution (FRU group), which is relatively close to the concentration in beverages and has been used in recent animal studies. In addition to the CON group (fed with tap water), a 10% (w/v) GLU group was set up in parallel which was different to the design of most previous studies.

It has been reported that maternal excess fructose consumption can result in glucose and lipid metabolism disorders in dams and offspring.[22,23] Similar to previous studies, we found that maternal high-fructose intake during pregnancy caused significantly increased TG concentrations in maternal and fetal serum. More importantly, AFGR was observed in the FRU group. Fructose intake usually causes caloric excess. Some studies have suggested that the adverse impact resulting from fructose intake was under hyper-caloric conditions.[1] In fact, the deleterious effect caused by fructose can also be found in the absence of excess energy intake.[24] In the present study, maternal daily total energy intake was similar during pregnancy in the three groups. Furthermore, previous studies found that high fructose provided to pregnant rats commonly caused decreased chow intake,

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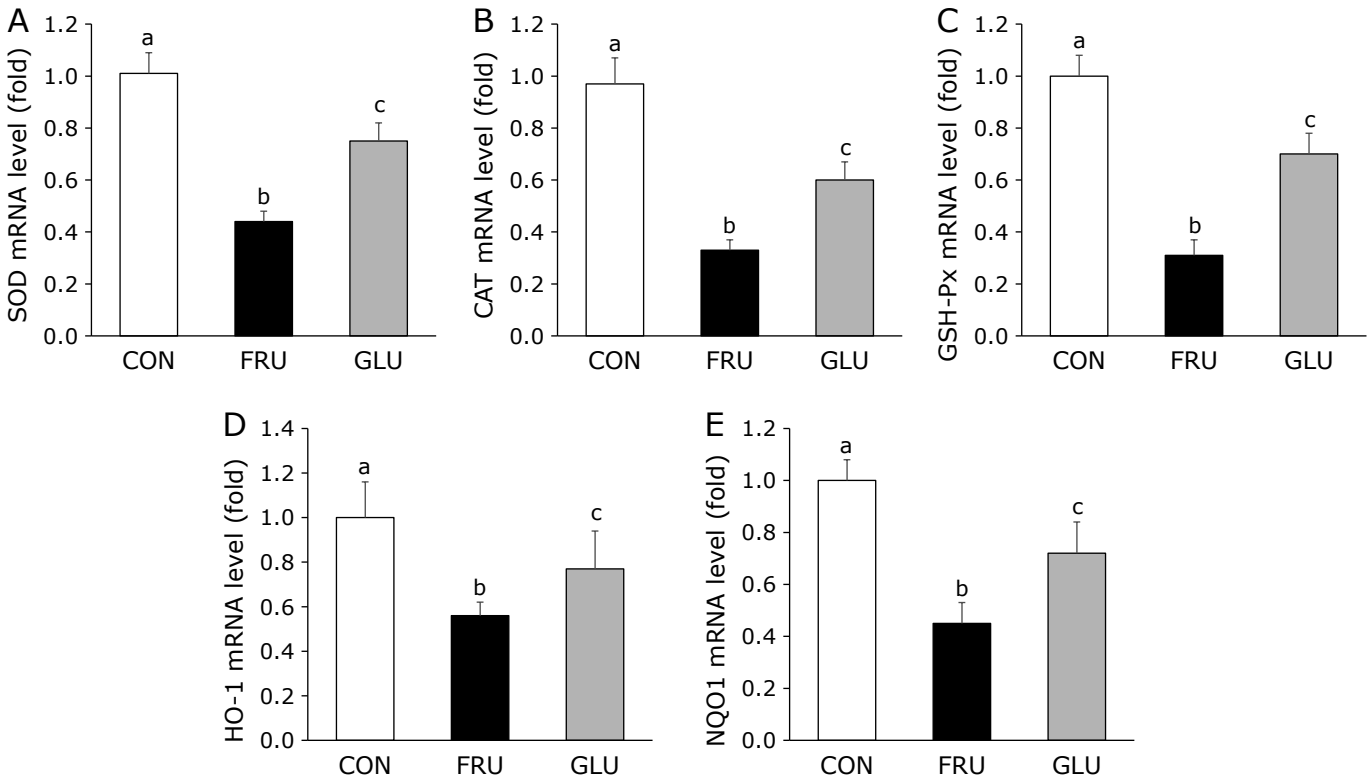

Fig. 5. Placental oxidative stress parameters mRNA expression levels. Values are normalized to β-actin and presented as means ± SD. Value of CON group has been set at 1. Values with different letters are significantly different from each other ($p<0.05$); $n = 10$ per group. CAT, catalase; CON, control; FRU, fructose; GLU, glucose; GSH-Px, glutathione peroxidase; HO-1, heme oxygenase-1; NQO1, NAD(P)H quinone oxidoreductase 1; SOD, superoxide dismutase.

leading to protein and micronutrients deficiency, which are known to be associated with adverse effects on offspring.[25] Indeed, significantly decreased chow consumption and increased fluid intake were observed in both the FRU and GLU groups in the present study suggesting a possible lower nutrients intake than that in the CON group, although there is no difference in daily total energy intake among the three groups. However, AFGR found in the FRU group cannot simply be explained by only nutritional deficits in dams as AFGR was not found in the GLU group.

The placenta responds to changes in maternal nutritional status and has a pivotal role in programming the fetal experience *in utero* due to adaptive changes in structure and function.[26] Fetal growth restriction is believed to be associated with placental insufficiency.[27] Decreased placental weight and low fetus/placenta ratio representative of placental insufficiency observed in the FRU group in the present study may be the reason for AFGR. Moreover, significantly decreased maternal serum PLGF with concomitant increased sFlt-1 concentration and an elevated sFlt-1/PLGF ratio in the FRU group further highlighted placental insufficiency. Similar results were also found in mice fed a high-fructose diet.[28] Maternal fructose intake in mice resulted in not only a low fetus/placenta ratio, but also a smaller labyrinth area in the placenta, influencing the maternal-fetal exchange and ultimately leading to fetal growth restriction.

Fructose can transport across the placenta and be present in fetal circulation.[13] In the present study, significantly increased serum fructose concentrations were found in dams and fetuses in the FRU group. The transport of fructose in most tissues mainly relies on the transporter. Glucose transporter 5 (GLUT5) is the sole transporter specific for fructose.[29] However, there are few conclusive data on the presence of GLUT5 in human placenta tissue. Consistent with previous studies,[30] placental GLUT5 mRNA expression was not detectable in the present study. Evidence has revealed that placental transport of fructose may be mediated by diffusion.[31] In addition, the placenta was found to be able to produce endogenous fructose.[32]

Fructose metabolism leads to elevated UA synthesis in the liver,[1] which also occurs in the placenta.[28] The UA formed in the placenta is considered to mediate the impact of fructose, promoting endothelial dysfunction and inefficient placentation.[33] Therefore, we detected the activity of enzymes involved in the fructose metabolism pathway and the concentration of UA in the placenta, and found that the activity of XOD and the concentration of UA in the FRU group were significantly elevated. Moreover, with increased serum fructose concentration, markedly increased UA concentrations were also found in maternal and fetal serum in the FRU group.

UA can act as an antioxidant extracellularly, but can induce oxidative stress intracellularly.[34] Oxidative stress may be the general underlying mechanism that links altered placental function to fetal programming.[26] During normal pregnancies, oxidants are necessary for normal development through cellular signaling. However, overproduction of ROS can lead to massive cellular damage, changing the course of pregnancy and generating a cascade effect that leads to the genesis of *in utero* programming of adult diseases.[14,35] In parallel with increased UA levels, a significant elevation in the lipid peroxidation product (MDA) concentration and decreased antioxidant enzymes (SOD, CAT, and GSH-Px) activities were found in maternal and fetal serum in the FRU group. Moreover, the FRU group had markedly lower antioxidant enzyme activities and mRNA expression levels in placentas.

Nrf2 is one of the most important transcription factors that

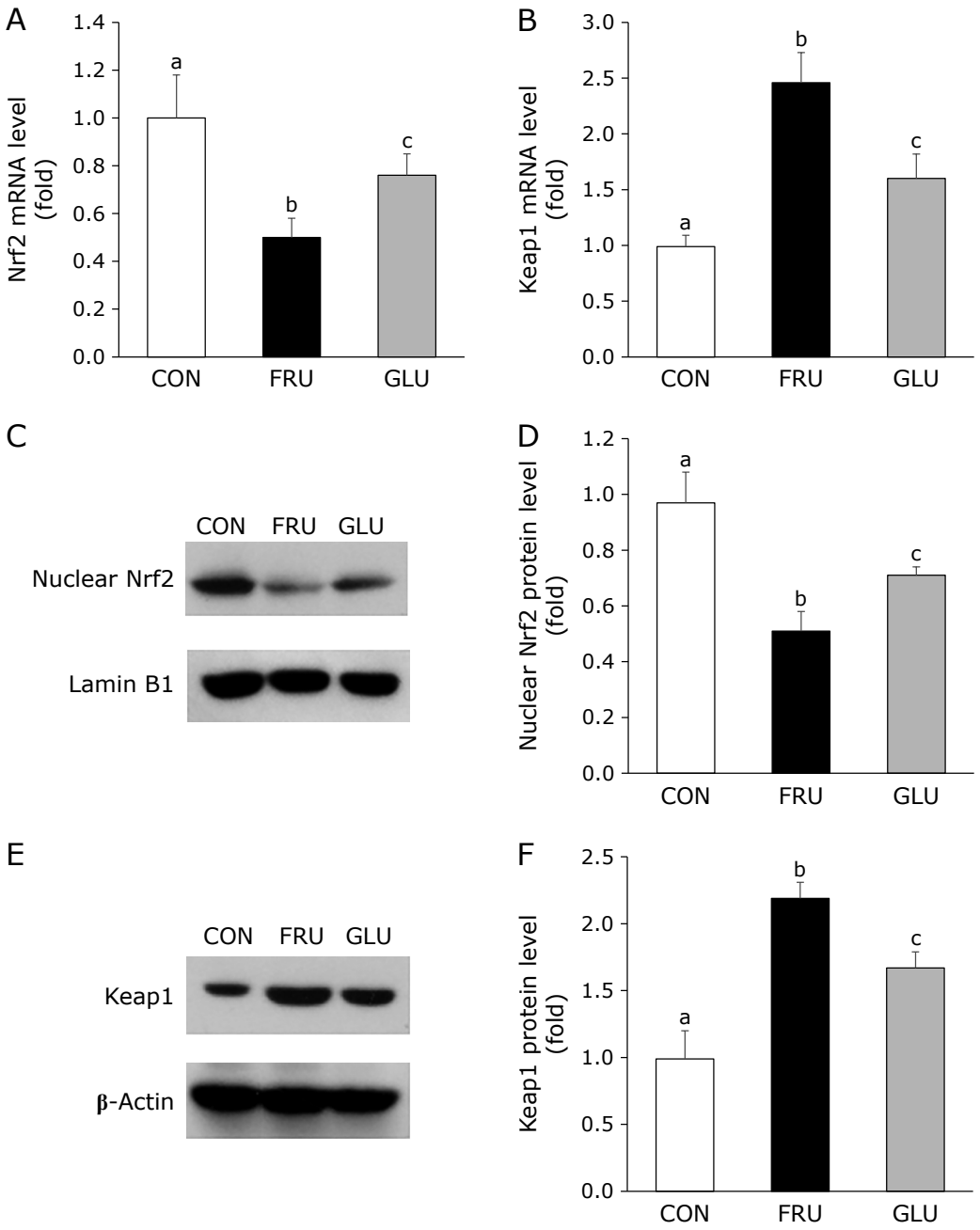

Fig. 6. Placental Nrf2 (A, D) and Keap1 (B, F) mRNA and protein expression levels. A representative photograph of the Western blot is shown (C, E). Values are normalized to β-actin and presented as means ± SD. Value of CON group has been set at 1. Values with different letters are significantly different from each other ($p<0.05$); $n = 10$ per group. CON, control; FRU, fructose; GLU, glucose; Keap1, kelchlike ECH-associated protein 1; Nrf2, nuclear factor erythroid 2-related factor 2.

regulates the cellular oxidative stress response.[36] Under quiescent conditions, Nrf2 is bound to Keap1, an adaptor protein for Cul3-based E3 ligase. During oxidative stress, Keap1 can be post-translationally modified and inactivated, which leads to decreased Nrf2 degradation and ultimately promotes Nrf2 translocation to the nucleus to activate its target genes.[36] The markedly decreased antioxidant enzymes detected in the FRU group in the present study (SOD, CAT, GSH-Px, HO-1, and NQO1) are all predominantly dependent on the Nrf2/Keap1 signaling pathway. Therefore, we determined the expression levels of Nrf2 and Keap1 mRNA and their respective proteins in the placentas in the three groups. It is possible that decreased nuclear Nrf2 was largely due to upregulated production of Keap1, finally resulting in the reduced expression of antioxidant enzymes. When the balance between the oxidants and antioxidative system is broken, oxidative stress increases and causes a series of negative impacts.

In conclusion, the present study demonstrates that maternal fructose intake during pregnancy results in the accumulation of UA and an imbalance of redox status in the placenta. Under this oxidative insult, the placenta is impaired leading to insufficiency, which ultimately induces AFGR. In light of the adverse effects on offspring health caused by maternal fructose intake during pregnancy, processed foods and beverages rich in fructose should be appropriately limited in pregnant women.

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Author Contributions

Study concept and design: HL and HZ; drafting of the manuscript: SL; critical revision of the manuscript: HL and HZ; acquisition and analysis of data: SL, BY, HZ, YW, and TG.

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Abbreviations

AFGR	asymmetrical fetal growth restriction
CAT	catalase
CON	control
ED	embryonic day
FG	fasting glucose
FIN	fasting insulin
FRU	fructose
GLU	glucose
GSH-Px	glutathione peroxidase
HO-1	heme oxygenase-1
Keap1	kelchlike ECH-associated protein 1
MDA	malonaldehyde
NQO1	NAD(P)H quinone oxidoreductase 1
Nrf2	nuclear factor erythroid 2-related factor 2
PLGF	placental growth factor
sFlt-1	soluble fms-like tyrosine kinase-1
SOD	superoxide dismutase
TG	triacylglycerol
UA	uric acid
XOD	xanthine oxidase

Conflict of Interest

No potential conflicts of interest were disclosed.

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