

Effect of high fructose consumption during pregnancy on fetal development and preliminary study on its mechanism

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Abstract: Excessive intake of fructose is linked to the development of type 2 diabetes and obesity. However, the effect of fructose consumption on fetal development during pregnancy is not well established. The aim of this study was examined the impact of the fructose intake during pregnancy on the placental and fetus development. Dams fed fructose throughout gestation leads to fetal weights was decreased as the dose of fructose intake increasing; Meanwhile, the number of absorbing birth and stillbirth was increased. This change was not observed in control group (CG) and normal fructose group (NFG). Fructose feeding had no effect on the rate of skeletal deformities and visceral hemorrhage. The placental weight was decreased in super high fructose group (SHFG); Serum fructose level was significantly increased in high fructose group (HFG) and SHFG. The placental lever of vascular endothelial growth factor (VEGF) was lower, whereas soluble fms-like tyrosine kinase-1 (sFlt-1) concentration is higher in HFG and SHFG. In conclusion, maternal high fructose intake during pregnancy increased the risk of stillbirth, absorbed fetus, and induced the toxicity of fetal development and results in vascularization of the placenta.

Keywords: Fructose; Fetal development; VEGF; sFlt-1

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1. Introduction

With the development of economy and the improvement of people's cognitive level, women's diet habit and life style have changed greatly during pregnancy[1]. Excessive energy intake during pregnancy is prone to obesity and diabetes and other diseases[2,3], pregnancy nutrition is one of the main factors that affect the health of pregnant women and fetal growth and development.

Epidemiological and experimental studies have revealed a significant relationship between maternal nutrition and metabolic consequences in offspring[4,5]. There is growing interest in the effects of adverse intrauterine conditions on the risk of obesity, diabetes and cardiovascular disease in the offspring. For instance, maternal under nutrition or overnutrition induces glucose intolerance, hypertension, obesity and insulin resistance in adult offspring[6-9].

Recent epidemiological and experimental studies have demonstrated that an increase in fructose consumption is paralleled by an increase in obesity, cardiovascular and type 2 diabetes[10,11]. Studies have demonstrated that dietary fructose during pregnancy and lactation induces renal programming, neuroendocrine dysfunction and overweight in adult offspring[12,13]. Nonetheless, the effects of high fructose consumption during pregnancy on fetal development involved placental remain unclear.

The placenta plays an important role in the mother-fetus relationship, maintaining fetal development. Changes in placental structure and function can trigger stillbirth and fetal growth

restriction (FGR)[14]; maternal hyperglycemia can lead to vascular disease, impaired placental function and fetal intrauterine hypoxia, which can affect fetal growth and development. Pregnancy from the implantation to the embryonic development[15] and the formation of the placenta to start delivery cannot be separated from the rich blood supply.

Fructose is one of the natural sugars found in fruits, and is sweeter than glucose and sucrose. Current related animal experiment, in the experimental design of fructose intake and normal eating habits are so far. Therefore, it is necessary to stimulate the situation of pregnant women with fructose intake, to give pregnant rats the same dose of fructose, to observe the effect of high fructose intake during pregnancy on fetal development, preliminary mechanism of the effect of the placenta.

2. Materials and Methods

2.1. Animal model

Ten-week-old female Sprague-Dawley rats were maintained at a constant temperature of 18~22 °C under a 12h light-dark cycle with ad libitum access to food and tap water. Adaptive feeding a week, two female rats was mated overnight with one male rat. The presence of spermatozooids in vaginal smears the following morning proved embryonic day (E)0. Pregnant rats were randomly divided into four groups: control group(CG; n = 8), normal dose of fructose group(NFG; n = 8), high dose of fructose group(HFG; n = 8) and super high dose of fructose group(SHFG; n = 8). The CG received 1ml distilled water through

gavages administration every day, the NFG, HFG and SHFG were given 1.6g/kg, 4.8g/kg and 8.0g/kg D-fructose solution through gavages administration during gestation. At E21, all dams were killed by anaesthetized, blood samples were collected, and then fetuses were removed, counted, weighed and position in uterine horn recorded, general observation of fetuses including stillbirth, fetal absorption and malformation etc. and then 4% formaldehyde fixed. Placental were dissected and weighed. All blood samples were centrifuged and the supernatant stored at -80°C for later analysis. Part of the placenta stored at -80°C after homogenate and another part of the 4% formaldehyde fixed until analysis.

2.2. Biochemical analysis

Part of the fetal was selected to examine skeletal abnormalities and some of the fetal were examined for internal organs placed in the Bouin fixation 1-2 weeks.

All blood samples were centrifuged at 2500rpm at 4 °C for 5 minutes and supernatants collected and stored at -20 °C until later analysis. Serum fructose concentration was analyzed using ultraviolet spectrophotometry.

Frozen Placenta tissue was homogenized and stored at -80 °C until later analysis. Placenta levels of sFlt-1 and VEGF were measured in duplicate using commercially available Enzyme-linked immunosorbent assay (ELISA) kits.

2.3. Statistical analysis

For bodyweights, organ weights, fructose, VEGF

and sFlt-1 the significance of differences between mean values was calculated using One-way analysis of variance, the mean differences between the two comparisons with LSD. Data were reported as mean ± standard deviation (SD) for continuous data. Stillbirth, absorbed fetus, skeletal deformity and visceral hemorrhage were performed using Chi-square test. Statistical analysis was performed using SPSS version 17.0 Statistical significance was accepted at $p < 0.05$ for all comparisons.

3. Results

3.1. Maternal and fetal characteristics

We observed in the maternal body weight increase by the end of the gestation (Table1), after 21 days of fructose administration. In comparison to the CG and NFG mothers, HFG showed an increase in the weight ($P < 0.05$) and SHFG rats showed marked increased in the weight ($P < 0.01$). Interestingly, SHFG group of their fetuses weight is lower than CG ($P < 0.05$). High fructose intake during pregnancy has no effect on number of fetal. In parallel, Compared with CG, the weight of placenta in SHFG groups was decreased (0.48 ± 0.16 vs 0.46 ± 0.12).

High fructose intake caused serious influence to the fetus (Table 2), Compared with CG and NFG, the fetal of HFG and SHFG have stillbirth and absorbed fetus ($P < 0.01$), fetus in SFHG have the most severe adverse outcomes. But skeletal deformity and visceral hemorrhage did not reappear.

Table 1 Maternal and fetal characteristics on Gestational Day (GD) 20

	CG	NFG	HFG	SHFG
Maternal body weight at Day 0 (g)	261.00±4.73	261.60±4.29	262.20±4.06	262.00±4.54
Maternal body weight at Day 20 (g)	308.89±5.08	315.70±3.32	320.6±2.55 ^a	340.00±7.97 ^{ab}
Number fetus/litter	10.44±0.90	9.70±0.92	8.60±0.91	10.88±0.92
Fetal body weight (g)	6.01±0.43	6.04±0.20	5.95±0.49	5.74±0.21 ^a
Placental weight (g)	0.48±0.16	0.47±0.14	0.46±0.33	0.46±0.12 ^a

Dams were given either distilled water (control) or fructose solution through gavages administration (fructose) throughout pregnancy. Data are the mean ± s.e.m. ^{ab} $P < 0.05$ compare d with CG and NFG.

Table 2 Fetal stillbirth and absorbed

group	live birth		stillbirth		absorbed fetus	
	n		n	%	n	%
CG	99		0		0	
NFG	92		0		0	
HFG	78		6	7.69 ^{ab}	4	5.12 ^{ab}
SHFG	76		10	13.16 ^{ab}	6	7.79 ^{ab}

Data are the mean ± s.e.m; ^{ab} $P < 0.05$ compare d with CG and NFG

3.2. Maternal serum fructose levels

Maternal fructose levels in CG and NFG dams were below (Figure 1). Maternal consumption in HFG and SHFG was significantly increased maternal serum fructose concentrations compared with CG and NFG ($P < 0.05$). Although maternal serum fructose of NG is higher than the CG, but no statistical significance compared two groups.

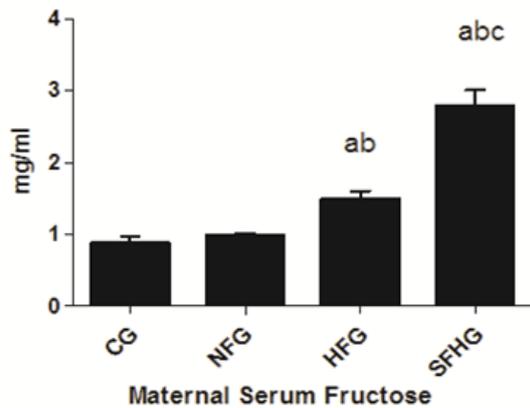


Figure 1. Maternal serum fructose levels. Data are means \pm SEM, n=8 per group. ^{abc} $P < 0.05$ compare d with CG and NFG.

3.3. VEGF and sFlt-1 levels in Placenta

Maternal Placenta levels of sFlt-1 and VEGF were compared at gestational 21 (Figure 2 and Figure 3). Placental levels of VEGF in SHFG were significantly lower than CG and NFG ($P < 0.01$), VEGF in HFG were also decreased ($P < 0.05$), while concentration of sFlt-1 were significantly higher in SHFG and HFG.

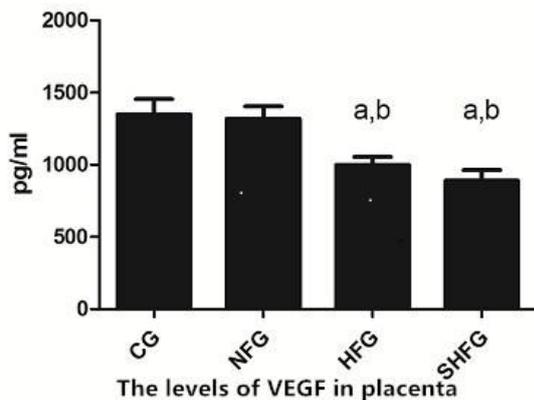


Figure 2. Levels of VEGF in maternal Placenta. Data are means \pm SEM, n=8 per group. ^{ab} $P < 0.05$ compare d with CG and NFG.

4. Discussion

High fructose consumption through pregnancy has been demonstrated to promote gestational diabetes (GM), hepatic steatosis, hypertension and glomerulosclerosis in rats as well as in humans[16-18].

Excessive fructose ingestion of pregnant dams affects spatial learning and memory in adult female offspring through Hippocampal brain-derived neurotrophic factor (BDNF) was decreased, induce renal programming and hypertension in adult male offspring[19]. Most previous experiments, swapping out fructose solution for drinking water or fructose instead of some or all of the carbohydrates, to observe the effects of high fructose intake on the body.; however, fructose intake in the design of the experiment is far from normal diet. Therefore it is necessary through simulation of fructose intake during pregnancy, give the pregnant rats equal doses of fructose, observe effects of high fructose consumption on the embryonic development, and discuss the influence of placenta during pregnancy on fetal growth and development.

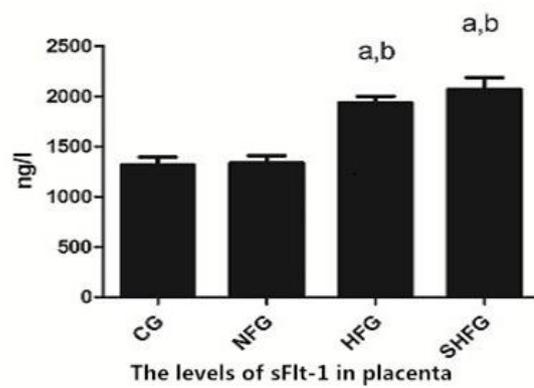


Figure3. Levels of sFlt-1 in maternal Placenta. Data are means \pm SEM, n=8 per group. ^{ab} $P < 0.05$ compare d with CG and NFG.

In the present study we have established that maternal additional fructose intake during pregnancy induced noticeably lower VEGF and elevated sFlt-1 in the placenta. The physiological consequences of this placental insufficiency included an increased frequency of placental ischemia and hypoxia and embryo deaths and absorbed in HFG and SHFG. Our data provide evidence that decreased VEGF and increased sFlt-1 in the placenta may contribute to the increased the occurrence of adverse outcome of fetal.

Consistent with previously published observations, we found that the bodyweight of fetal were gradually decreased and the maternal weight was increased with the increasing of the dosages of fructose[20]. One explanation for this observation could be that high fructose intake causes the increase of very low density lipoprotein (VLDL) levels and promotes the increase of fat deposition and body mass, the lipid deposition in visceral adipose tissue is also due to high fructose leads to triglyceride (TG)[21]. We have shown that fructose intake during pregnancy resulted in increased maternal serum fructose levels, the increase in body weight induced by high fructose intake may also be

related to the specific metabolic absorption process of fructose, after fructose consumption does not cause hypothalamic feeding regulation of the central site of the blood flow reduction, will not cause a sense of fullness. At the same time, fructose is a kind of energy substances, most of the food and drinks containing fructose, usually as a result of excess energy intake of the body, it is easy to cause the increase in body mass. Our unpublished data demonstrate that that excess fructose intake could lead to increased blood glucose levels in pregnant rats, which is consistent with the study of Yuuka Mukai [22], suggesting that fructose consumption during gestation low birth weight may lead to abnormal glucose metabolism related to fructose intake. High blood glucose make the maternal lost a lot of sugar from the urine, so will use their own fat and protein, the fetus cannot get enough nutrition raw material supply, resulting in a reduction in body mass[23,24].

According to the 'Developmental Origins of adult Health and Disease' (DOHaD) concept, adverse environment during the developmental program may affect the long-term health and increased susceptibility to various chronic diseases of the offspring[25]. In the present study showed that the stillbirth and absorbed fetus in HFG and SHFG in addition to the fetus weight loss, but there was no occurrence of visceral hemorrhage and skeletal deformities in experiment. In this study, the level of VEGF in placenta was decreased while sFlt-1 levels were increased. We hypothesized that the maternal and the fetus of adverse outcome may be associated with the altered development of the placental vasculature. Embryonic development is characterized by the growth of the blood vessels in the fetus, the placenta and the endometrium. Vascularization and angiogenesis is the placenta build endometrial decidual vascular network and the basis of the embryo implantation, immersion, rich vascular network of fetal growth and development, tissue differentiation and maternal material exchange between provides the basic guarantee. In recent years, studies have found that VEGF was associated with the development of angiogenesis in the endometrium, placenta and fetal growth and development, and fertilization, implantation and implantation. VEGF through the placenta to regulate the process of pregnancy, in the early pregnancy, VEGF activity played a crucial role[26]. The expression change of VEGF may give rise to abnormal embryonic angiogenesis obstacle, leading to the occurrence of abortion[27]. VEGF binds to two tyrosine kinase (TK) receptors, VEGFR-1 (Flt-1) and VEGFR-2 (KDR/Flk-1), VEGFR-1 (sFlt-1) functions as an endogenous VEGF inhibitor, sFlt-1 is a high affinity of VEGF receptors[28]. sFlt-1 is mainly secreted by syncytiotrophoblast[29], sFlt-1 can antagonize the VEGF, resulting in insufficient vascularization degree of placenta tissue[30], affect the integrity of vessel wall and the permeability of vessel. We believe that

this can be explained by the high dose of fructose made an impact on the vascular of the placenta.

The current study is characterized by decreased in the fetal weight, as well as an increase in the number of stillborn pups and absorbed fetus; Pregnancy is the process of the embryo and fetus in utero growth, placenta provides adequate blood and a nutrient is the assurance to making a successful progress. Therefore, the formation of the placental blood capillary net plays an important role in fetal growth and development. VEGF are found in villous trophoblast, villous blood vessels and villous stroma. Early in pregnancy, VEGF in a paracrine secretion function of VEGF receptors in placental vascular endothelial cells to promote the development of placental vascular network, when the late pregnancy angiogenesis decreased, VEGF play the role of vascular permeability, maintain the maternal-fetal nutrient exchange[31]. In most experiments, the relationship between sFlt-1 and pre-eclampsia was studied, and the research on the adverse outcomes of the offspring was less. The level of sFlt-1 in the serum of fetal growth restriction patients was higher than that in the control group[32]. The result of this study suggest that the decreased of the expression of VEGF and the increased of sFlt-1 in placenta, sFlt-1 excessive expression, competition binding of free VEGF and levels of VEGF decreased, reducing angiogenesis, trophoblast cells decreased synthesis, trophoblast on the invasiveness of uteroplacental arteries is abnormal, uteroplacental blood flow reduction, maternal-fetal nutrition impairment, make fetal obtain nutrients decrease. Based on the above reasons may be the cause of the occurrence of fetal death and other adverse outcomes.

5. Conclusion

Despite high fructose intake during pregnancy does not appear offspring visceral hemorrhage and skeletal deformities such as serious consequences, but with the increase of fructose dose is stillbirth and absorbed fetus. With the change of the maternal cognitive behavior, the consumption of fruit increased dramatically. Through the experiment, might increase public awareness of the hazards of excessive fructose, as per capita daily intake of fructose is safe to provide some reference to safe, we advises no more than 400 grams for people, especially maternal need to more strict limits the intake of high fructose, to improve the perinatal outcomes and reduce the unreasonable diet during pregnancy adverse effects on the children's health.

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