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Original Research

Maternal fructose consumption alters messenger RNA expression of hippocampal StAR, PBR, P450 (11 β), 11 β -HSD, and 17 β -HSD in rat offspring



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ABSTRACT

Hippocampal functions such as neuronal protection and synapse formation are positively modulated by neurosteroids, which are synthesized *de novo* within the brain. However, the mechanisms regulating neurosteroidogenesis remain unclear. Fructose, which is used as a sweetener, affects steroid hormone synthesis in peripheral endocrine organs. This monosaccharide can penetrate the blood-brain barrier and impair hippocampal function. Also, fructose is secreted into milk and is thus delivered to the fetus. Based on these observations, we hypothesized that the hippocampal neurosteroidogenesis in the offspring may be affected by maternal fructose consumption. Female rats were fed with normal water or 20% fructose solution during gestation and lactation. Maternal calorie intake did not change significantly, and no significant change in body weight was observed. The levels of messenger RNAs (mRNAs) for steroidogenic enzymes and proteins in the hippocampus of the offspring were analyzed by real-time reverse transcriptase polymerase chain reaction. Maternal fructose consumption during gestation and lactation increased mRNA levels of P450(11 β)-2, 11 β -HSD-2, and 17 β -HSD-1 in the offspring hippocampus, and reduced levels of mRNAs for StAR, PBR, and 17 β -HSD-3. Maternal fructose consumption might influence hippocampal neurosteroidogenesis in offspring.

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Abbreviations: 17 β -HSD, 17 β -hydroxysteroid dehydrogenase; CYP11B, cytochrome P450 having 11 β -hydroxylation activity; P450 (17 α), cytochrome P450 having steroid 17 α -hydroxylation and C17-C20 side-chain cleavage activity; StAR, steroidogenic acute regulatory protein.

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1. Introduction

Fructose is a natural sugar found in fruits. Because of its low cost and high sweetness, this compound is used in beverages throughout the world, and its consumption has increased gradually in the United States since the 1950s [1]. Epidemiologic studies suggested that fructose consumption is associated with the incidence of obesity and type 2 diabetes [2]. Moreover, fructose is considered to be a risk factor for dementia and cognitive dysfunction [3]. Animal studies have shown that fructose consumption reduces hippocampal neurogenesis [4], suggesting an association between fructose consumption and brain physiologic activity. (See [Tables 1](#) and [2](#).)

It has been suggested that maternal nutrition status can alter the phenotype of the offspring [5,6]. Maternal fructose consumption induced low birth weight, suggesting a potential effect of maternal fructose on fetal development [7]. Also, maternal fructose overconsumption during pregnancy and lactation affected glucose intolerance and fatty liver in the offspring [6]. More recently, an effect of maternal fructose consumption on brain activity was reported. Maternal overconsumption of fructose induced brain mitochondrial dysfunction [8] and caused a state resembling metabolic syndrome in the brain [8]. Taken together, these results indicate that maternal fructose consumption may affect not only fetal development but also brain function in the offspring.

Neurosteroids are synthesized *de novo* from cholesterol in the nervous system [9]. Neurosteroids play important roles in brain function. For example, the neurosteroid allopregnanolone promotes the proliferation of rodent neural progenitor cells [10]. This steroid also stimulates neuron survival in the hippocampus [11]. Other neurosteroids, such as corticosterone and estradiol, enhance spine formation, indicating that neurosteroids are essential for brain normal activity [12]. The rat hippocampus possesses active steroidogenic proteins and enzymes, such as steroidogenic acute regulatory protein (StAR), cytochrome P450 17 α -hydroxylase/c17,20-lyase (P450[17 α]), and 17 β -hydroxysteroid dehydrogenase (17 β -HSD; [Fig. 1](#) [13,14]. We have studied the regulatory mechanism of hippocampal neurosteroid synthesis. We previously reported that tributyltin, an environmental pollutant, and 9-*cis*-retinoic acid regulate hippocampal neurosteroid synthesis [14,15]. We also clarified that social isolation stress stimulates hippocampal estradiol synthesis [13]. Based on our previous observations, hippocampal neurosteroid synthesis appears to be modified by internal and external stimuli.

Because neurosteroids are important for the brain, disturbance of this synthesis may result in impaired brain activity.

Given that fructose modifies steroid hormone synthesis [16,17], we hypothesized that hippocampal neurosteroidogenesis in offspring may be affected by maternal fructose consumption. Therefore, we assessed whether or not maternal fructose consumption affects hippocampal neurosteroid synthesis. Female rats were fed fructose solution during gestation and lactation, and messenger RNA (mRNA) for hippocampal neurosteroidogenic molecules in male offspring was quantified by real-time polymerase chain reaction (PCR). Here, we showed that maternal fructose consumption during pregnancy and lactation alters mRNA levels for StAR, PBR, 17 β -HSD-3, 17 β -HSD-1, P450(11 β)-2, and 11 β -HSD-2.

2. Methods and materials

2.1. Animals

Animals were handled in accordance with the Regulations for the Management of Laboratory Animals at Fujita Health University. The protocol for the ethical use of these animals was approved by the Animal Care and Use Committee at Fujita Health University (Permit No. H0862). Wistar rats were obtained from Japan SLC (Hamamatsu, Japan) and maintained under standard conditions on a 12-hour light/dark cycle, with regular rat chow available *ad libitum*. Upon confirmation of gestation by presence of a vaginal plug, female rats (aged 2 months) were assigned to receive either normal water (control group, *n* = 2) or 20% fructose solution (fructose group, *n* = 2), as reported previously [18]. Fructose solution or water was administered from day 1 of pregnancy to postnatal day 21 (the end of the weaning period). The fructose administration period was 43 to 45 days. Male offspring born in the control group (*n* = 6) and the fructose group (*n* = 9) were killed just after weaning (postnatal day 21), and the hippocampus was isolated. In some experiments, female rats were used to measure calorie intake and body weight during gestation and lactation (control group, *n* = 6; fructose group, *n* = 7) described as previously [18].

2.2. General anesthesia

A rodent anesthesia chamber was used. Anesthesia was induced and maintained using isoflurane [19]. A surgical plane of anesthesia was confirmed when a toe pinch failed to elicit a change in respiratory rate or pattern.

2.3. Total RNA isolation and complementary DNA synthesis

Total RNA was isolated from the hippocampus with TRIzol reagents (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Total RNA concentration was determined using a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) [19]. Complementary DNAs were prepared using the M-MLV Reverse Transcriptase (Invitrogen) with a Random Primer (TaKaRa, Otsu, Japan). Approximately 1 μ g of total RNA was reverse transcribed and used for real-time PCR analysis.

Table 1 – Ingredient composition of MF diet

Ingredients (g/kg)	MF diet
Casein	200
Corn starch	150
Corn oil	v50
Sucrose	500
Mineral Mix	v35
Vitamin Mix	v10
Choline bitartrate	2
DL-Methionine	v3
MF diet : moderate-fat diet.	

Table 2 – Change of body weight, food intake and solution intake of pregnant rats

Gestation period		Day 1	Day 3	Day 5	Day 7	Day 9	Day 11	Day 13
Body weight (g)	Control	208 ± 7	215 ± 5	226 ± 7	229 ± 9	238 ± 7	249 ± 8	264 ± 9
	Fructose	210 ± 12	218 ± 15	227 ± 13	236 ± 11	243.99 ± 10.50	255.17 ± 9.98	267.62 ± 12.66
Food intake (g)	Control		24.4 ± 5.3	34.2 ± 16.5	29.5 ± 1.7	32.5 ± 0.5	33.6 ± 38	34.5 ± 2.3
	Fructose		20.0 ± 3.8	21.4 ± 2.9	20.2 ± 2.6	21.8 ± 3.5	22.6 ± 3.7	23.9 ± 4.7
Solution intake (mL)	Control		32.4 ± 8.1	54.8 ± 27.9	42.0 ± 10.9	52.0 ± 8.6	50.0 ± 4.69	58.4 ± 13.7
	Fructose		56.4 ± 28.2	67.2 ± 34.4	60.8 ± 14.8	60.0 ± 20.1	64.4 ± 18.4	70.4 ± 29.5

2.4. Quantitative real-time PCR analysis

Real-time PCR analysis was performed using the 7500 Real-Time PCR system (Applied Biosystems, Carlsbad, CA, USA) in a total reaction mixture volume of 10 μ L containing 5 μ L of Power SYBR Green PCR Master Mix (Applied Biosystems) [20]. Comparative threshold cycle values were used to determine relative mRNA expression levels, as described previously [20]. Real-time PCR results were normalized by GAPDH expression levels [14,15]. Polymerase chain reaction primers were listed in a previous report [21].

2.5. Statistical analyses

2.5.1. Analysis for calorie intake and body weight

Calorie intake was calculated by food and solution intake, which was recorded daily during gestation (days 1–13). Body weight was also measured daily during gestation (days 1–13). Two-way mixed-measures analysis of variance was performed on the body weight data for the factors of water (normal and fructose) and housing period.

2.5.2. Quantitative real-time PCR analysis

For quantitative real-time PCR analysis, amounts of each mRNA in the normal and fructose groups were examined using the two-tailed unpaired *t* test, as reported previously [21]. The

statistical software package SPSS ver. 11.5 (IBM, Armonk, NY, USA) was used [21]. The criterion for significance was $P < .05$. All results are expressed as means \pm SEM.

3. Results

We calculated maternal calorie intake during gestation (days 1–13). Greater solution intake was observed in fructose group dams, whereas dietary intake was decreased (data not shown), as compared with the group receiving normal water. No significant difference was observed between the normal and fructose groups (Fig. 2, left); the body weight was also the same (Fig. 2, right). Fructose-induced obesity was not observed. Therefore, we did not conduct a pair feeding study.

We examined the effect of maternal fructose consumption on mRNA levels for hippocampal neurosteroidogenic molecules using quantitative real-time reverse transcriptase PCR (Fig. 3). The amounts of mRNAs for StAR, PBR, and 17 β -HSD-3 in the offspring hippocampus were reduced by maternal fructose consumption. In contrast, the up-regulation of mRNA levels for P450(11 β)-2, 11 β -HSD-2, and 17 β -HSD-1 was observed. The levels of other mRNAs examined remained unchanged. Reduced mRNA levels for P450(17 α) were observed, but the difference were not significant. These data indicate that maternal fructose consumption during pregnancy and lactation affects the transcription of steroidogenic molecules in the rat hippocampus.

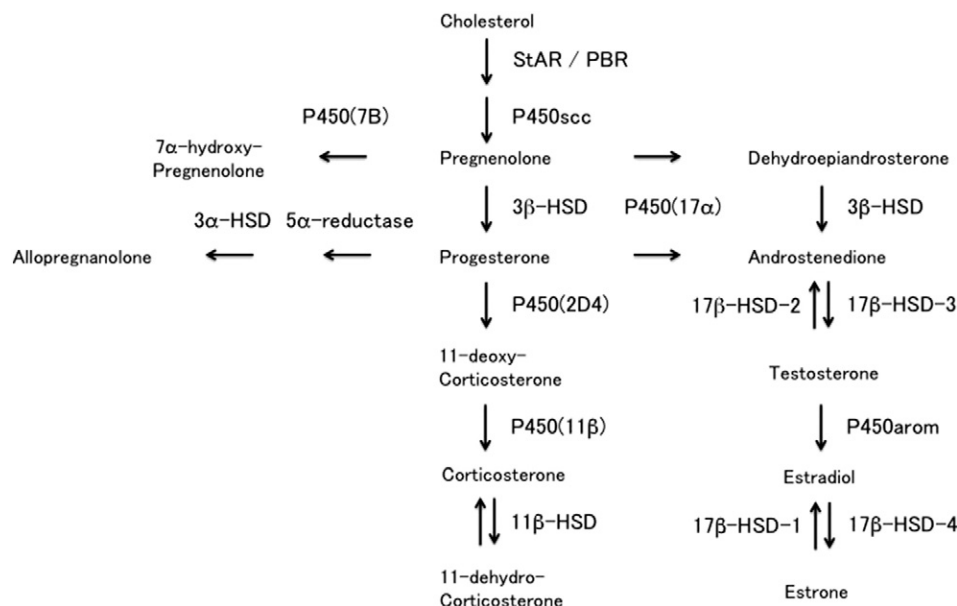


Fig. 1 – Steroid synthesis pathway in the rat hippocampus. Arrows indicate specific steps involving individual enzymes and proteins.

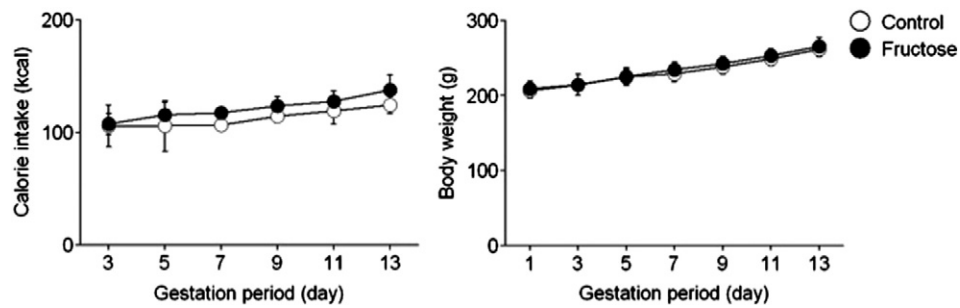


Fig. 2 – Calorie intake was calculated based on food and solution intake, which was recorded daily during gestation (days 1–13, $n = 6$; left). Body weight was also measured daily during gestation (days 1–13; right). No significant difference in calorie intake or body weight was observed between the normal ($n = 6$) and fructose ($n = 7$) groups. Fructose-induced obesity was not observed. Data are expressed as mean \pm SEM.

4. Discussion

The present study using real-time PCR provides evidence on the relationship between maternal fructose consumption and

steroidogenesis in the offspring of rat hippocampus. We analyzed the effect of maternal fructose consumption on the levels of mRNAs for several steroidogenic molecules. Among mRNAs encoding steroidogenic enzymes and proteins in the offspring hippocampus, those for StAR, PBR, 17β -HSD-1, 17β -

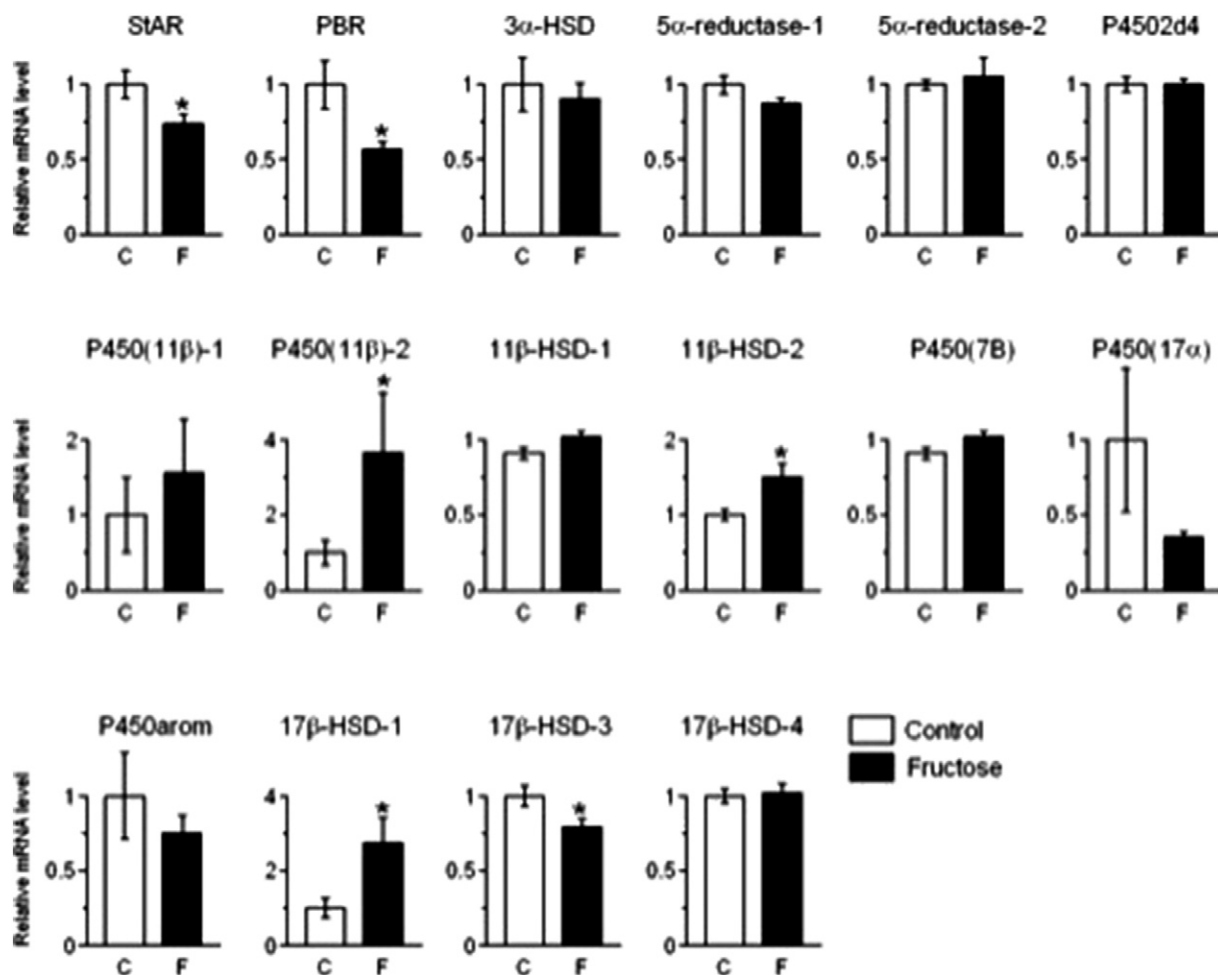


Fig. 3 – Real-time PCR of mRNA levels of neurosteroidogenic enzymes and proteins in the rat hippocampus. The amounts of mRNA in the offspring of fructose group (black bars, $n = 9$) are shown as a relative value to those of the control group (white bars, $n = 6$). mRNA levels of StAR, PBR and 17β -HSD-3 were decreased only in the offspring of the fructose group, whereas increased levels of mRNA for P450(11 β)-2, 17β -HSD-1, and 11 β -HSD-2 were observed in both groups. The other mRNA levels remained unchanged. Columns and error bars represent means \pm SEM. * $P < .05$.

HSD-3, P450 (11 β)-2, and 11 β -HSD-2 showed altered levels after maternal fructose consumption. This phenomenon was caused by maternal fructose consumption during gestation and lactation, suggesting that maternal nutritional status affects hippocampal neurosteroid synthesis in the offspring. To our knowledge, this study is the first to demonstrate a relationship between maternal fructose consumption and brain steroidogenesis. Maternal fructose consumption might disturb hippocampal neurosteroidogenesis and affect hippocampal function, such as learning memory, in the offspring.

The effect of excess maternal nutrition on the risk of disease in the offspring has received much attention. It is well-known that maternal nutritional status affects the development of disease in the offspring [5,6,22]. Maternal obesity predisposes the offspring to the development of metabolic disorders [6,7]. Fructose-induced obesity was not observed in the present study. No significant differences in maternal calorie intake during gestation and lactation were observed between groups receiving water only and 20% fructose solution, and no differences in body weight were observed. Therefore, we considered that the alterations in mRNA levels for neurosteroidogenic enzymes were due to fructose consumption, not calorie intake. In addition, our unpublished data demonstrate that the offspring born from dams receiving fructose had insulin resistance and impaired glucose tolerance (at age 160 days), suggesting that fructose consumption during gestation and lactation has a harmful effect on offspring development. This phenomenon also appears to be independent of calorie intake.

Messenger RNAs for StAR and PBR are relatively highly expressed in the hippocampus and are rate-limiting steps in steroid hormone synthesis [13]. PBR interacts with StAR and promotes steroidogenesis [23]. In addition, 17 β -HSD-3 is essential for sex steroid synthesis and its activity is demonstrated in studies using the rat hippocampus [24]. Our previous studies demonstrated that hippocampal neurosteroidogenesis is regulated by transcriptional alteration of steroidogenic molecules. In particular, we reported significant correlations between the StAR mRNA level and hippocampal estradiol concentration [13]. Furthermore, changes in P450(17 α) mRNA affected the estradiol level in the hippocampus [14]. Based on this observation, reductions in levels of mRNAs for steroidogenic molecules caused by maternal fructose consumption may result in a reduced level of hippocampal estradiol. An increased level of mRNA for 17 β -HSD-1 may not contribute to compensation in the hippocampal estradiol level; the hippocampal 17 β -HSD-1 activity was found to be relatively low [25]. Moreover, the level of the substrate of 17 β -HSD-1 (estrone; 0.015 nM) was much lower than the estradiol level (8.4 nM) in the hippocampus [25].

Estrogen is pivotal in processes such as synapse formation, neuroprotection, and neurogenesis in the hippocampus [26]. In studies using adult rat hippocampal slices, estradiol was found to rapidly enhance long-term depression in CA1, CA3, and the dentate gyrus, as well as the density of spines on pyramidal neurons in CA1 [27]. Estradiol regulates the morphology of astrocytes and the expression of brain-derived neurotrophic factor [26]. In addition, estradiol appears to protect the brain against injury because it reduces cell death in response to various noxious stimuli, such as oxidative stress [11]. Estradiol treatment was observed to enhance spatial memory by what is

considered to be stimulation of hippocampal function [26]. Given the current results, the positive effects of estradiol may be reduced in the hippocampus of pups of dams that consume a large amount of fructose. During lactation (the postnatal period), the hippocampus synthesizes estradiol actively [28]. Also, it has been reported that endogenous estradiol synthesized in the hippocampus has neuroprotective effects. Specifically, a blockade of hippocampal estradiol synthesis increases apoptosis, whereas hippocampus-derived estradiol prevents neuronal loss by excitotoxic damage, demonstrating the importance of hippocampus-derived estradiol in neuroprotection [29,30]. Maternal fructose intake may interrupt the normal hippocampal function of pups due to a reduction in the brain estradiol level.

The present study also demonstrates the possibility that maternal fructose may affect hippocampal corticosterone synthesis. Changes in levels of mRNAs for P450(11 β)-2 and 11 β -HSD-2 were observed after maternal fructose intake, suggesting that the hippocampal glucocorticoid level may be regulated by maternal fructose. De novo hippocampal corticosterone synthesis was demonstrated recently. This steroid induces rapid spinogenesis via synaptic glucocorticoid receptors and kinase networks in the hippocampus [31]. Maternal fructose intake may affect hippocampal function by disrupting hippocampal glucocorticoid synthesis.

Fructose has been reported to have neurotoxic effects. Rafati et al [32] reported that high fructose solution induced neuronal loss in the rat brain. In the rat hippocampus, reduced neurogenesis was observed after feeding with fructose solution for 4 weeks [4], and a high-fructose diet impaired spatial memory in male rats [3]. In contrast, neurosteroid inhibited neuronal cell loss and stimulated neurogenesis and spatial memory [11,26,30]. The present study demonstrated changes in mRNA levels for neurosteroidogenic molecules after maternal fructose consumption. Thus, the levels of neurosteroids in the hippocampus of the offspring may be changed by maternal nutrition status. Adverse effects of fructose in the hippocampus may be exerted by the changes in hippocampal steroid levels, at least partially.

In this study, we only analyzed the effect of maternal fructose intake on mRNA levels. The effect on neurosteroidogenic enzyme level and hippocampal neurosteroid levels was not examined. The impact on offspring behavior of altered neurosteroidogenesis due to maternal fructose intake remains unclear. Thus, further detailed investigations are needed to clarify the effect of maternal fructose consumption on hippocampal neurosteroid synthesis in offspring.

In conclusion, although the effect of maternal fructose consumption on developing metabolic disorders in the offspring has been studied, the effect on the central nervous system remains unclear. We showed that maternal fructose consumption might be at least partially responsible for adverse effects in their offspring's hippocampus. Considering the high rates of fructose consumption throughout world, the adverse effect of maternal fructose intake on brain function should be examined in detail.

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