Effects of High-Fructose Corn Syrup Intake on Glucocorticoid Metabolism in Rats During Childhood, Adolescence and Adulthood

Authors

Yuki Nouchi[1, 2], Eiji Munetsuna[3], Hiroya Yamada[4], Mirai Yamazaki[5], Yoshitaka Ando[1], Genki Mizuno[1, 2], Ryosuke Fujii[2], Itsuki Kageyama[1, 2], Takuya Wakasugi[1], Tomohide Sakakibara[1], Atsushi Teshigawara[1], Hiroaki Ishikawa[1], Yohei Shimono[3], Koji Suzuki[2], Shuji Hashimoto[4], Koji Ohashi[1]

Affiliations

1 Department of Informative Clinical Medicine, Fujita Health University School of Medical Sciences, Toyoake, Aichi, Japan
2 Department of Preventive Medical Sciences, Fujita Health University School of Medical Sciences, Toyoake, Aichi, Japan
3 Department of Biochemistry, Fujita Health University School of Medicine, Toyoake, Aichi, Japan
4 Department of Hygiene, Fujita Health University School of Medicine, Toyoake, Aichi, Japan
5 Department of Medical Technology, Kagawa Prefectural University of Health Sciences, Takamatsu, Kagawa, Japan

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Georg Thieme Verlag, Rüdigerstraße 14,
70469 Stuttgart, Germany

Correspondence

Eiji Munetsuna
Department of Biochemistry, Fujita Health University School of Medicine
Toyoake
Aichi
Japan
Tel: + 81-6-6293-2451
mntneiji@fujita-hu.ac.jp

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ABSTRACT

The consumption of high-fructose corn syrup (HFCS) has been increasing in recent decades, especially among children. Some reports suggest that children and adolescents are more sensitive to the adverse effects of fructose intake than adults. However, the underlying mechanism of the difference in vulnerability between adolescence and adulthood have not yet been elucidated. In this study, we attempted to elucidate the different effects of HFCS intake at different growth stages in rats: childhood and adolescence (postnatal day (PD) 21–60), young adulthood (PD60–100), and adulthood (PD100–140). Since alterations in hepatic glucocorticoid (GC) metabolism can cause diseases including insulin resistance, we focused on GC metabolizing enzymes such as 11 beta-hydroxysteroid dehydrogenase 1 and 2 (Hsd11b1 and Hsd11b2) and steroid 5 alpha-reductase 1 (Srd5a1). Western blotting showed an increase in Hsd11b1 expression and a decrease in Hsd11b2 expression in childhood and adolescence but not in adulthood. We also observed changes in Hsd11b1 and Hsd11b2 activities only in childhood and adolescence, consistent with the results of mRNA and protein expression analysis. The effect of high-fructose intake with regards to GC metabolism may therefore vary with developmental stage. This study provides insight into the adverse effects of fructose on GC metabolism in children in the context of increasing rates of HFCS consumption.

ABBREVIATIONS

HFCS	high-fructose corn syrup
GC	glucocorticoid
Hsd11b1, 2	11 beta-hydroxysteroid dehydrogenase1, 2
Srd5a1	steroid 5 alpha-reductase 1
PD	postnatal day

Introduction

Fructose is a monosaccharide found in fruits and vegetables and is often used in foods and beverages as high-fructose corn syrup (HFCS). The consumption of HFCS increased rapidly in the 1970s owing to its sweet taste and low price. Epidemiological and experimental evidence suggests that fructose ingestion is a risk factor for metabolic diseases, such as obesity, hypertension, dyslipidemia, and insulin resistance [1–3]. Recently, HFCS consumption has increased, especially in children [4]. Vos et al. showed that nearly one-fourth of the adolescent population obtain more than 15 % of their calories from fructose [5]. Moreover, rates of adolescent obesity have been on the rise in recent years, from 1 % in 1975 to 8 % in 2016 [6]. Thus, there is a concern that HFCS intake during adolescence may contribute to increasing rates of certain metabolic disorders.

Adolescence is the period of organ development and physical growth from childhood to adulthood, defined as 10–19 years of age in humans and as postnatal days (PD) 21–60 in rodents [7]. The importance of the adolescent environment is becoming clearer; a growing body of evidence indicates that children and adolescents are more sensitive than adults to the effects of food intake/nutrition, such as fructose consumption. Hsu et al. showed more significant effects of fructose intake on brain function, metabolic outcomes, and neuroinflammation in adolescence than in adulthood [8]. In addition, impaired insulin signaling because of high-fructose intake has been observed during adolescence but not during adulthood [8, 9]. However, the causes of the difference in vulnerability between adolescence and adulthood has not yet been elucidated.

Glucocorticoids (GCs; corticosterone in rodents, cortisol in humans) are hormones important for the regulation of lipid and glucose metabolism and blood pressure [10, 11]. GCs are synthesized in the adrenal gland, and their synthesis is regulated by the hypothalamic-pituitary-adrenal axis. Synthesized GCs are regulated by 11 beta-hydroxysteroid dehydrogenase 1, 2 (Hsd11b1, Hsd11b2) and the steroid 5 alpha-reductase 1 (Srd5a1) enzymes [12, 13]. Hsd11b2 is mainly expressed in the kidney, while Srd5a1 is expressed in the liver [14, 15], and these enzymes convert active GC to inactive form [12, 13]. Hsd11b1 is mainly expressed in the liver and skeletal muscle, and regenerates active GC from its inactive form [16].

The alterations in the expression of GC metabolizing enzymes disrupt GC action and can cause disorders in the affected tissues [16]. For example, Hsd11b1 overexpression in the liver causes hepatic dysfunction [17], the deletion of Srd5a1 in mice causes insulin resistance and hepatic steatosis [14], and kidney-specific Hsd11b2 knock-out in mice leads to hypertension [18].

Fructose intake alters the expression of enzymes involved in GC metabolism [19, 20]. London et al. showed that high-fructose diets increased hepatic expression of Hsd11b1 [19]. Likewise, Vasiljević et al. reported that fructose-induced increase in Hsd11b1 expression may lead to disruption of insulin signaling [20]. These studies suggest the induction of abnormal GC metabolism due to fructose intake. Given that fructose intake before adulthood is harmful, fructose-induced metabolic abnormalities in GC may consequently be more pronounced in children and adolescents than in adulthood. However, previous studies have not considered the effects of fructose intake on individuals at different stages of maturation [19, 20]. To better understand the mechanism, fructose-induced disease pathogenesis needs to be analyzed based on the developmental stage.

In this study, we aimed to test the effects of 20 % HFCS solution (containing 11 % fructose) on GC metabolism at different developmental stages of experimental rats, including childhood and adolescence (PD21–60), young adulthood (PD60–100), and adulthood (PD100–140).

Materials and Methods

Animals

This study was approved by the Animal Ethics Committee of Fujita Health University (Permit No. H0862). Male Sprague-Dawley (SD) rats (SLC, Shizuoka, Japan) were obtained from Japan SLC (Hamamatsu, Japan) and kept under standard conditions at room temperature (23 °C ± 3 °C) under a 12:12 h light-dark cycle (the light period started at 8:00 am). In animal studies using rodents, adolescence is indicated as the period from weaning (PD21) to PD60. The period from PD60 to PD100 is defined as young adulthood [21]. In this study, the period after young adulthood was defined as adulthood. We divided the experimental period into three parts: childhood and adolescence (PD21–60; Period I), young adulthood (PD60–100; Period II), and adulthood (PD100–140; Period III) [22]. The control group (C, n = 7–8) received distilled water for 40 days, whereas the HFCS-fed group (H, n = 7–8) received 20 % HFCS solution for the same period. A 20 % aqueous HFCS solution was prepared using 75 % HFCS (Japan Corn Starch, Tokyo, Japan). Standard chow (MF; Oriental Yeast, Tokyo, Japan) and water were available *ad libitum* to all animals throughout the experimental period. According to data from the Third United States National Health and Nutrition Examination Survey, the mean fructose consumption was > 10 % of total caloric intake and constituted approximately 20 % of total caloric intake in the top 5 % of fructose consumers [5]. As fructose is most commonly consumed in the form of HFCS, this study modeled conditions that relatively substituted the modern human diet. The body weight of the rats was measured every two weeks. At the end of the experimental period, the rats were fasted for 6 h before being perfused with saline and dissected under isoflurane anesthesia. The kidney, adrenal gland, gastrocnemius muscle, and liver were harvested and stored at −80 °C until use.

Quantification of mRNA expression

Total RNA was isolated from the kidney, adrenal gland, gastrocnemius muscle, and liver using TRIzol Reagent (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions [23]. Quantitative polymerase chain reaction (qPCR) was performed using the QuantStudio 7 Flex system (Thermo Fisher Scientific, Waltham, MA, USA) and the Thunderbird SYBR qPCR Mix (Toyobo, Osaka, Japan).

Conditions were as follows: 95°C for 1 min and 40 cycles of 95°C for 15 s, 55°C for 30 s, and 72°C for 1 min. The target gene primers were designed by Fasmac Co., Ltd. (Kanagawa, Japan) and have been described previously [24]. The expression levels of target genes were normalized to the mRNA levels of *actin beta* (*Actb*) as an internal control. We calculated the fold changes in the expression between the control and HFCS-fed groups using the $2^{-\Delta\Delta Ct}$ method [25].

Western blotting

The kidney, adrenal gland, gastrocnemius muscle, and liver were homogenized in RIPA buffer (Wako Pure Chemicals, Osaka, Japan). In brief, the extracted protein were boiled in EzApply Buffer (Atto, Tokyo, Japan) for 3 min. Sodium dodecyl sulfate-treated proteins (20 µg) were separated by electrophoresis on a 12.5 % polyacrylamide gel (Atto, Tokyo, Japan) and transferred onto membranes. (Atto, Tokyo, Japan) The membranes were incubated overnight at 4°C with primary antibodies against Hsd11b1 (ab39364; Abcam, Cambridge, UK), Hsd11b2 (14192–1-AP; Proteintech, Rosemont, USA), Srd5a1 (ab110123; Abcam, Cambridge, UK), and beta-actin (ab8227; Abcam, Cambridge, UK) [26]. Subsequently, the membranes were incubated with horseradish peroxidase-conjugated secondary antibody for 1 h at room temperature. Immunoreactive bands were visualized using Immobilon Western Chemiluminescent HRP Substrate (Millipore, Billerica, MA, USA). The intensities of specific chemiluminescence bands were analyzed using a Lumino image analyzer (ImageQuant LAS 3000; GE Healthcare, Amersham, UK).

Activities of 11 beta-hydroxysteroid dehydrogenase 1 and 2 (Hsd11b1and Hsd11b2) enzymes

Liver was homogenized to 40 mg/mL concentration in 30 mM Tris buffer (pH = 7.4) containing 0.9 mM ethylenediaminetetraacetic acid and 0.3 mM sucrose. Reaction buffers A and B were then prepared. The activity of Hsd11b1 or Hsd11b2 was determined in buffer A or B, respectively. Reaction buffer A was 0.01 M sodium phosphate buffer containing 200 µM cortisone (Merck, Darmstadt, Germany) and 2 mM NADPH. Reaction buffer B contained 0.01 M sodium phosphate buffer containing 100 µM cortisol (VWR International Ltd., Leuven, Belgium) and 2 mM NAD^+. Cortisone and cortisol were used as substrates for Hsd11b1 and Hsd11b2 because these steroids show high affinity with rat Hsd11b1 and Hsd11b2 [27, 28]. The reaction was initiated by adding the homogenate (equivalent to 10 mg of tissue). After incubation at 37°C for 30 min, the reaction buffer was extracted by adding 2 mL chloroform and 50 µM dexamethasone (Merck, Darmstadt, Germany) as an extrinsic control. The chloroform phase was transferred to a tube and evaporated at 45°C to dryness. The residue was dissolved in 60 % methanol and fractionated using a high-performance liquid chromatography (HPLC) system (UV-2075 Intelligent UV/Vis Detector, Jasco Inc., Tokyo, Japan) to measure cortisone, cortisol, and dexamethasone [29]. The reverse-phase HPLC analysis was performed on a $5C_{18}$-MS-II column (Nacalai Tesque, Kyoto, Japan) at a column temperature of 30°C and detection wavelength of 254 nm.

Statistical analysis

Statistical analyses were performed using JMP version 14 (SAS Institute, Cary, NC, USA). All data are expressed as the mean ± standard deviation, and statistical analysis was performed using the Student's t-test [30]. Statistical significance was defined as $p < 0.05$.

Results

The experimental rats were fed distilled water or 20 % HFCS solution for 40 days during childhood and adolescence (PD21–60), young adulthood (PD60–100), and adulthood (PD100–140) (▶ **Fig. 1**). Assessment of the effects of HFCS intake on body weight during each developmental period showed no significant differences between these periods (▶ **Fig. 1**).

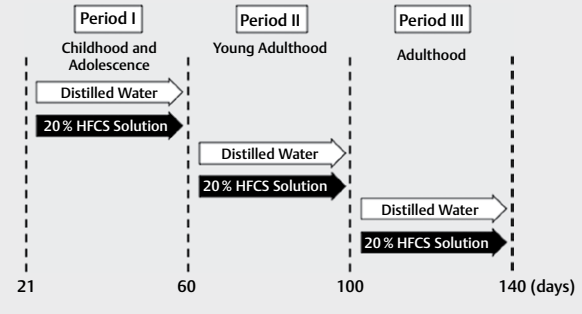
To evaluate the effect of excess HFCS intake on GC metabolism in childhood and adolescence, we analyzed the mRNA expression of *Hsd11b1* and *Hsd11b2,* and *Srd5a1* in the kidney, adrenal gland, muscle, and liver. Renal *Hsd11b2* levels were lowered during Period I (▶ **Table 1**). In the liver, *Hsd11b1* levels increased, and levels of *Hsd11b2* and *Srd5a1* decreased during Period I. Hepatic *Hsd11b1* and *Hsd11b2* expression was not significantly different in Periods II and III, and *Srd5a1* expression decreased during all periods (▶ **Fig. 2**).

We also quantified Hsd11b1, Hsd11b2, and Srd5a1 protein levels in these tissues. The kidney, adrenal gland, and muscle did not show any significant changes (▶ **Table 2**). Following hepatic mRNA expression, Hsd11b1 protein levels increased significantly, and Hsd11b2 protein levels decreased significantly during Period I ($p = 0.05$), but the levels did not differ significantly during Periods II and III (▶ **Fig. 3**). In addition, hepatic Srd5a1 protein levels decreased during Period I, but no difference was observed during Periods II and III (▶ **Fig. 3**).

We analyzed the activities of Hsd11b1 and Hsd11b2 enzymes that are likely to be highly sensitive to GC metabolism in each period (▶ **Fig. 4**). As expected, Hsd11b1 enzyme activity increased while that of Hsd11b2 enzyme decreased during Period I, but no difference was observed during Periods II and III. The enzymatic activity of hepatic Hsd11b1 and Hsd11b2 were 10.4 ± 2.5 and 11.0 ± 0.5 pmol/min/mg tissue in the control group, respectively.

Discussion

We analyzed the effects of HFCS intake on GC metabolism in the kidney, adrenal gland, muscle, and liver at different developmen-

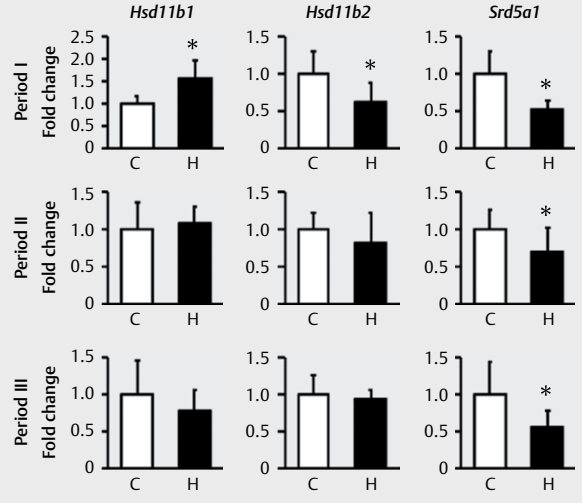


▶ **Fig. 1** Experimental design. The experimental period was divided to assess rats in three stages of their growth: childhood and adolescence (postnatal day (PD) 21–60; Period I), young adulthood (PD60–100; Period II), and adulthood (PD100–140; Period III). Male rats were fed either distilled water or a 20 % HFCS solution for 40 days.

▶ **Table 1** List of qPCR analysis of corticosterone metabolizing enzyme.

	Period I		Period II		Period III	
	C	H	C	H	C	H
mRNA	Relative mRNA Level		Relative mRNA Level		Relative mRNA Level	
Liver						
Hsd11b1	1.00 ± 0.16	1.57 ± 0.38*	1.00 ± 0.36	1.09 ± 0.22	1.00 ± 0.45	0.78 ± 0.27
Hsd11b2	1.00 ± 0.31	0.62 ± 0.27*	1.00 ± 0.23	0.83 ± 0.39	1.00 ± 0.26	0.95 ± 0.11
Srd5a1	1.00 ± 0.29	0.51 ± 0.12*	1.00 ± 0.27	0.69 ± 0.34*	1.00 ± 0.43	0.56 ± 0.22*
Kidney						
Hsd11b1	1.00 ± 0.29	0.73 ± 0.26	1.00 ± 0.12	1.02 ± 0.28	1.00 ± 0.15	1.13 ± 0.27
Hsd11b2	1.00 ± 0.23	0.52 ± 0.09*	1.00 ± 0.22	0.98 ± 0.20	1.00 ± 0.22	0.88 ± 0.28
Srd5a1	1.00 ± 0.23	0.77 ± 0.40	1.00 ± 0.13	0.92 ± 0.21	1.00 ± 0.16	0.91 ± 0.26
Adrenal Gland						
Hsd11b1	1.00 ± 0.23	1.75 ± 0.86	1.00 ± 0.23	1.00 ± 0.25	1.00 ± 0.42	0.99 ± 0.23
Hsd11b2	1.00 ± 0.31	0.94 ± 0.11	1.00 ± 0.24	1.02 ± 0.23	1.00 ± 0.26	1.24 ± 0.19
Srd5a1	1.00 ± 0.20	1.43 ± 0.61	1.00 ± 0.17	0.97 ± 0.22	1.00 ± 0.40	1.16 ± 0.26
Muscle						
Hsd11b1	1.00 ± 0.46	0.81 ± 0.21	1.00 ± 0.30	1.17 ± 0.43	1.00 ± 0.22	1.10 ± 0.21
Hsd11b2	1.00 ± 0.50	1.06 ± 0.42	1.00 ± 0.64	0.61 ± 0.38	1.00 ± 0.79	1.15 ± 0.25
Srd5a1	1.00 ± 0.31	0.73 ± 0.40	1.00 ± 0.50	1.40 ± 0.57	1.00 ± 0.41	0.74 ± 0.18

Values are presented as mean ± standard deviation. *Abbreviations:* 11 beta-hydroxysteroid dehydrogenase 1, 2 (Hsd11b1, Hsd11b2); steroid 5 alpha-reductase 1 (Srd5a1). C: control group; H: HFCS-fed group. HFCS: high-fructose corn syrup $^*p<0.05$.



▶ **Fig. 2** Effect of HFCS intake on hepatic mRNA levels during each period. *Hsd11b1*, *Hsd11b2*, and *Srd5a1* mRNA levels were quantified by quantitative polymerase chain reaction. The results are expressed as the ratio of the relative intensity of the gene expression levels to that of *Actin beta*. C, control group (n = 7–8); H, HFCS-fed group (n = 7–8). Values are presented as mean ± standard deviation. $^*p<0.05$.

tal stages. HFCS-induced increase in hepatic Hsd11b1 activity was observed only in the liver in Period I. Similarly, the reduction of hepatic Hsd11b2 activity was also induced only in Period I. Thus, our study shows that the effects of HFCS in relation to GC metabolism may vary with the developmental period.

The significant effects of GC exposure are reported more in adolescence than in adulthood. For example, Kinlein et al. observed chronic GC exposure-induced reduction in bone density in adolescence but not in adulthood [31]. According to our enzyme activity analysis, GC did not seem to accumulate in the liver during Periods II and III, although its accumulation may increase during Period I. Adverse effects of high-fructose intake were only observed in adolescence [8, 9]. This phenomenon may be partially explained by the presence or absence of abnormal hepatic GC metabolism.

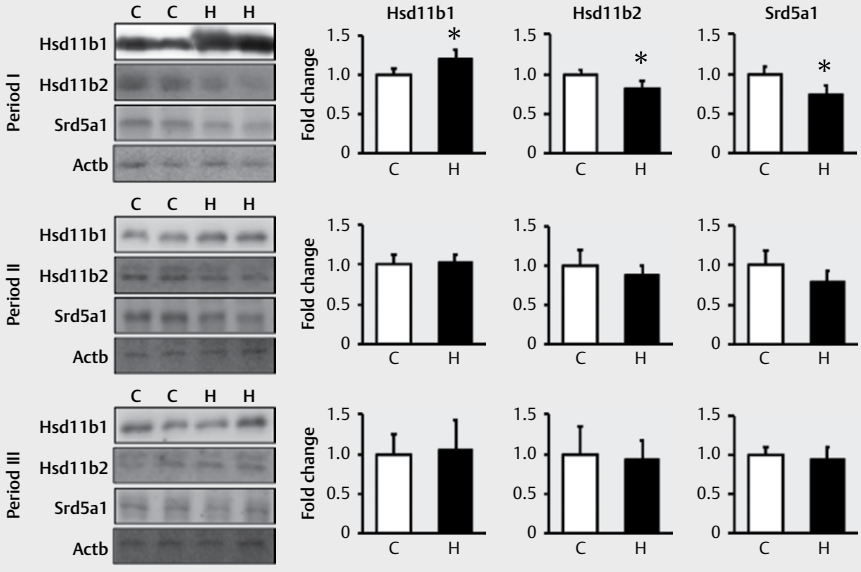
In this study, we observed increased Hsd11b1 activity in the rat liver in Period I but not in Periods II and III. Liver-specific Hsd11b1 overexpressing mice display insulin resistance and dyslipidemia [17], while Hsd11b1 knock-out mice are protected from metabolic disorders [32]. These reports may support our finding that accumulated active GC in the liver exacerbates the adverse effects of fructose in adolescence. We observed reduced hepatic Hsd11b2 activity by approximately 50 % after HFCS intake during Period I. Hsd11b2 is mainly expressed in the kidney; however, Chia et al. showed that Hsd11b2 enzyme activity in the liver is about 70 % of that in the kidney [33]. The decrease in Hsd11b2 activity in the liver may contribute to the increase in active GC. Moreover, we observed a reduction in hepatic Srd5a1 activity in Period I. In one study, the affinity of the Srd5a1 enzyme for GC was 1000-fold lower than that of Hsd11b2 [34, 35], implying that the decrease in Srd5a1 activity may not contribute to GC inactivation compared to Hsd11b2 or has only a negligible effect.

Our results implied that adolescents are more vulnerable to environmental changes than adults and are more likely to experience negative consequences. However, several animal experiments have shown that adverse effects of fructose intake occur not only in adolescence but also in adulthood. This discrepancy in findings may be explained by differences in the concentration of fructose used.

▶ **Table 2** List of western blotting analysis of corticosterone metabolizing enzymes.

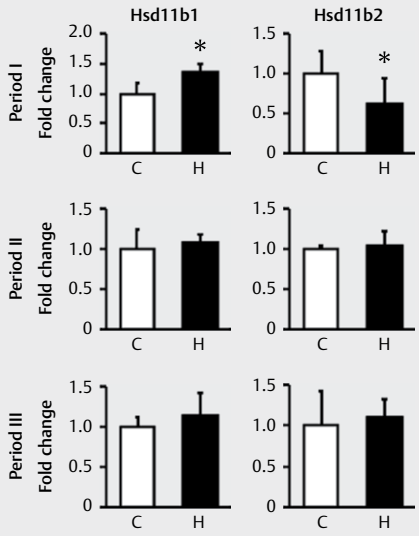
	Period I		Period II		Period III	
	C	H	C	H	C	H
Protein	Relative Protein Level		Relative Protein Level		Relative Protein Level	
Liver						
Hsd11b1	1.00 ± 0.09	1.20 ± 0.12*	1.00 ± 0.14	1.04 ± 0.09	1.00 ± 0.26	1.04 ± 0.39
Hsd11b2	1.00 ± 0.06	0.82 ± 0.10*	1.00 ± 0.20	0.88 ± 0.13	1.00 ± 0.34	0.93 ± 0.25
Srd5a1	1.00 ± 0.10	0.73 ± 0.13*	1.00 ± 0.18	0.79 ± 0.13	1.00 ± 0.09	0.93 ± 0.16
Kidney						
Hsd11b1	1.00 ± 0.27	0.97 ± 0.35	1.00 ± 0.07	1.01 ± 0.14	1.00 ± 0.17	0.98 ± 0.07
Hsd11b2	1.00 ± 0.25	1.01 ± 0.27	1.00 ± 0.04	1.01 ± 0.11	1.00 ± 0.31	0.97 ± 0.26
Srd5a1	1.00 ± 0.23	1.11 ± 0.31	1.00 ± 0.21	1.09 ± 0.20	1.00 ± 0.23	0.85 ± 0.15
Adrenal Gland						
Hsd11b1	1.00 ± 0.08	0.99 ± 0.41	1.00 ± 0.35	0.96 ± 0.20	1.00 ± 0.30	1.02 ± 0.40
Hsd11b2	1.00 ± 0.19	0.95 ± 0.19	1.00 ± 0.27	1.00 ± 0.22	1.00 ± 0.18	1.04 ± 0.19
Srd5a1	1.00 ± 0.21	1.09 ± 0.20	1.00 ± 0.03	0.96 ± 0.10	1.00 ± 0.22	0.94 ± 0.12
Muscle						
Hsd11b1	1.00 ± 0.21	0.99 ± 0.15	1.00 ± 0.20	0.83 ± 0.28	1.00 ± 0.25	1.17 ± 0.29
Hsd11b2	1.00 ± 0.34	1.01 ± 0.14	1.00 ± 0.32	1.04 ± 0.22	1.00 ± 0.39	1.25 ± 0.39
Srd5a1	1.00 ± 0.41	1.14 ± 0.29	1.00 ± 0.28	0.82 ± 0.41	1.00 ± 0.33	0.90 ± 0.26

Values are presented as mean ± standard deviation. *Abbreviations*: 11 beta-hydroxysteroid dehydrogenase 1, 2 (Hsd11b1, Hsd11b2); steroid 5 alpha-reductase 1 (Srd5a1). C: Control group; H: HFCS-fed group. HFCS: high-fructose corn syrup *$p < 0.05$.



▶ **Fig. 3** Effect of HFCS intake on hepatic protein levels during each period. Hsd11b1, Hsd11b2, and Srd5a1 protein levels were quantified using western blotting. Representative samples of western blot images are shown. The results were expressed as a ratio of the relative intensity of protein expression to that of Actin beta. C: control group (n = 4–6); H: HFCS-fed group (n = 4–6). Values are presented as mean ± standard deviation. *$p < 0.05$.

Some studies regarding the adverse effects of fructose have been conducted using 60–70 % fructose concentrations [36, 37], which is an unlikely concentration to be ingested as part of a modern diet. Another possible explanation is the difference in the duration of the intake period. We administered the HFCS solution for 40 days, unlike some studies in which adult rats were fed 10 % fructose for a longer time [38, 39]. For example, Ibrahim et al. reported that adult rats fed a 10 % fructose solution in drinking water for 16 weeks showed increased serum glucose and insulin levels [38]. Notably, these reports have observed body weight gain, which differs from the results of our study (**Supplemental Fig. 1**). In summary, the lack of significant effects of HFCS during adulthood in this study



▶ **Fig. 4** Effect of HFCS intake on hepatic Hsd11b1 and Hsd11b2 enzyme activities during each period. Hepatic Hsd11b1 and Hsd11b2 enzyme activities according to HFCS intake are shown. Hsd11b1 activity was calculated based on the rate of conversion of cortisone to cortisol. Hsd11b2 activity was calculated based on the rate of conversion of cortisol to cortisone. These metabolites were measured using high-performance liquid chromatography. C: control group (n = 5–7); H: HFCS-fed group (n = 4–7). Values are presented as mean ± standard deviation. $^{*}p < 0.05$.

may be because of differences in the concentration of fructose administration and length of the intake period.

According to our previous studies, exposure to fructose during early life stages such as fetal and lactation induces changes in miRNA expression and DNA methylation level in the liver [23, 40]. These epigenetic modifications may be maintained at least until adulthood, altering mRNA expression of genes related to lipid metabolism. These reports suggest that the effects on GC-related gene expression changes may be observed via epigenetic changes even after HFCS intake is discontinued. Further study is needed to analyze this point.

The current study has a few limitations. First, we did not clarify the mechanism of the transcriptional changes in GC metabolizing enzymes in adolescent HFCS intake. Cooper et al. reported that interleukin-1 beta (Il1b) and tumor necrosis factor-alpha (TNFa) decrease Hsd11b2 and increase Hsd11b1 expression and activity [41]. Therefore, we analyzed *Il1b* and *TNFa* mRNA levels but did not observe any increase in their levels (data not shown). Further analysis is needed to determine the mechanism of the change in GC metabolizing enzymes in adolescent HFCS intake. Next, this study only analyzed GC metabolism in the liver and not steroid levels. Fructose intake increases GC levels, but given that increased GC in the liver is associated with abnormal lipid metabolism and insulin resistance [42], it is necessary to measure steroid levels.

In conclusion, we showed that HFCS intake during adolescence may induce hepatic GC accumulation. This study provides insight into the adverse effects of fructose on GC metabolism in children in the context of increasing rates of HFCS consumption.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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