Transporter Gene Expression and Transference of Fructose in Broiler Chick Intestine

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Recent studies have suggested that a high-fructose diet leads to the development of metabolic syndrome in mammals. However, relatively little information is available regarding the absorption of fructose in the chicken intestine. We therefore investigated fructose absorption and its transporters in the chicken small intestine. The gene expression of three transporters (glucose transporter protein member 2 and 5 and sodium-dependent glucose transporter protein 1) in the jejunum of fasted chicks were lower than those in chicks fed *ad libitum*. The everted intestinal sacs (*in vitro* method for investigating intestinal absorption) showed that the concentration of fructose uptake rapidly increased within 15 min after incubation, and then gradually increased until 60 min. After 15 min of incubation, fructose uptake in the *ad libitum* chick intestine was approximately 2-fold that in the fasted intestine and was less than half of the glucose uptake in the *ad libitum* chick intestine. Our results suggest that fructose is absorbed in the small intestine of chicks and that uptake is decreased by fasting treatment with decreases in the mRNA expression of related transporters.

Introduction

Fructose is classified as a monosaccharide of hexose, as is glucose, but fructose is a ketose whereas glucose is an aldose. Additionally, the digestion and absorption of fructose and glucose show some differences (Patel *et al.*, 2015). Although both fructose and glucose are transported from enterocytes into the blood via glucose transporter protein member 2 (GLUT2) (Thorens *et al.*, 1990), fructose is absorbed into the cytosol by GLUT5 (Kayano *et al.*, 1990) and glucose is transported through the apical membrane by sodium-dependent glucose transporter protein 1 (SGLT-1) (Wood and Trayhurn, 2003). A schematic illustration of these process is shown in Fig. 1. As with mammals, these glucose transporter isoforms (GLUT2, GLUT5, and SGLT-1) have been identified in chickens (Wang *et al.*, 1994; Garriga *et al.*, 1999; Garriga *et al.*, 2004), and fructose and glucose appear to use the same transporter mechanisms to cross the membrane of the chicken small intestine (Kimmich and Randles, 1975; Ferrer *et al.*, 1994; Garriga *et al.*, 1997; Garriga *et al.*, 2004). However, few studies have examined

the detailed mechanism of fructose absorption in chickens.

Several methods have been utilized to evaluate intestinal absorption. The everted gut sac method was originally developed by Wilson and Wiseman (1954) and has been widely applied to investigate the transport of various substances (e.g., amino acids, sugars, and drugs) across the epithelium of the intestine, (Turner *et al.*, 1990; Garriga *et al.*, 1997; Barthe *et al.*, 1998; Kleinow *et al.*, 2006; Hamilton and Butt, 2013). Transport into the sac can be measured gravimetrically, which is advantageous because the mucosal surface for transport can be relatively large (Hamilton and Butt, 2013).

To clarify the absorption of fructose in the chicken small intestine, we measured the transporter gene expression and fructose uptake using the everted sacs of chick intestine and compared the results with those of glucose. The stage beginning at 4 days of age is the adaptive early stage from yolk dependence to digestion of feed (Noy and Sklan, 1998), and a better understanding of absorption during this period may improve the later growth and health of chickens.

Materials and Methods

Animals

Day-old male broiler chicks (Chunky) were obtained from a local hatchery (Fukuda Hatchery, Okayama, Japan). Birds were maintained in a room with 24-h lighting and a temperature of 30℃. They were given free access to a com-

mercial starter diet (Nichiwa Sangyo Co. Ltd., Kobe, Japan) and water during the pre-experimental period. Chicks (4 days old) were distributed into experimental groups based on their body weights so that the average body weight was as uniform as possible for each treatment. The birds were reared individually in experimental cages up to the time of the experiments. The handling of the birds was performed in accordance with the regulations of the Animal Experiment Committee of Hiroshima University.

Gene Expression of Transporters

Chicks were divided into two groups: fed *ad libitum* and 24-h fasted. The fasting period was determined according to previous reports (Shiraishi *et al.*, 2011). At the end of each treatment period, tissue samples (jejunum) from chicks under each condition were collected and snap-frozen in liquid nitrogen and then stored at −80℃ until RNA isolation.

RNA was isolated from the dissected tissue using Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. To rule out the possibility that PCR products resulted from amplification of genomic DNA contaminating the RNA sample, RNA samples were treated

Fig. 1.   **Model for transporters across enterocytes showing the brush-border GLUT5 and SGLT-1 transporters and basolateral GLUT2 in the small intestine.** GLUT5 mediates entry of fructose into enterocytes by facilitated diffusion, while GLUT2 facilitates exit of glucose and fructose from epithelial cells. SGLT1 is responsible for glucose uptake.

with DNase I using a DNA-free kit (Ambion, Austin, TX, USA). Total RNA (500 ng) was reverse-transcribed at 42℃ for 15 min in 10 μL of 1× Prime Script RT Enzyme Mix I (Takara, Shiga, Japan). The reaction product was subjected to real-time PCR according to the instructions for the Light Cycler system (Roche Applied Science, Basel, Switzerland). Briefly, following a denaturation step at 95℃ for 10 s, PCR was carried out with a thermal protocol consisting of 95℃ for 5 s and 60℃ for 20 s in 20 μL buffer containing 1×SYBR Premix EX Taq (Takara) and 0.2 μM of each primer. Primers (GLUT2, GLUT5, SGLT-1, and RPS17) were designed according to Gilbert *et al.* (2007) and are shown in Table 1. To normalize the data, ΔC_T was calculated for each sample by subtracting the C_T of RPS17 from the C_T of the gene of interest. For relative quantitation, ΔC_T for the defined control group was subtracted from the ΔC_T of each experimental sample to generate $\Delta\Delta C_T$. The $\Delta\Delta C_T$ was then used to calculate the approximate fold-difference, $2^{-\Delta\Delta CT}$. The results were expressed as the gene of interest mRNA/ RPS17 mRNA ratio. There were 7 and 5 chicks in the *ad libitum* and fasting groups, respectively.

Transfer of Fructose by Sacs of Everted Small Intestine

The incorporation of monosaccharide into everted intestine sacs was determined as described by Hamilton and Butt (2013) with some modifications. Briefly, birds were anesthetized and a segment of the jejunum quickly removed, rinsed with ice-cold Krebs-Henseleit buffer (KHB), everted, and cut into 8.0-cm lengths. The everted sac filled with a serosal solution (1 mL of KHB) was placed inside a plastic tube containing the mucosal solution (20 mL of KHB with 5 mM of fructose or glucose), which was bubbled continuously with 95% O_2 at 39℃ in an oscillating water bath. At each time point after incubation, the sac was removed from the flask, its surface was drained, and the fluid contents were recovered and weighed using an electric digital balance with a precision of ±1 mg. Samples of initial monosaccharide solution and final serosal and mucosal fluids were analyzed for fructose or glucose. The concentrations of fructose and glucose were measured using a commercial kit (F-kit; R-Biopharm, Darmstadt, Germany). To determine the time course of fructose uptake, the incubation time for fructose uptake in *ad libitum* chicks was 15, 30, or 60 min. For comparison, we also examined fructose uptake in fasted chicks and glucose uptake in *ad libitum* chicks at 15 min, when lower levels of nutrient transporters may have been present (Pento and Mousissian, 1988). The number of chicks in each group was as follows: fructose (*ad libitum*) at 15 min,
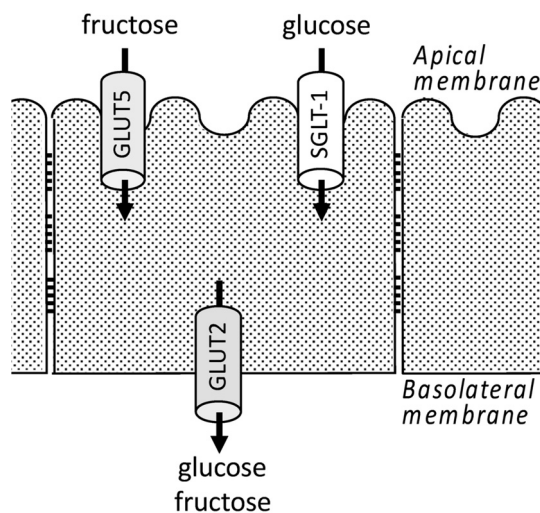
Table 1.   **Oligonucleotide primer sequences for real-time PCR**

Primer	Forward (5′→ 3′)	Reverse (5′→ 3′)	Accession No.
RPS17	AAGCTGCAGGAGGAGGAGAGG	GGTTGGACAGGCTGCCGAAGT	NM_204217
GLUT2	CACACTATGGGCGCATGCT	ATTGTCCCTGGAGGTGTTGGTG	Z22932
GLUT5	TTGCTGGCTTTGGGTTGTG	GGAGGTTGAGGGCCAAAGTC	XM_417596
SGLT-1	GCCATGGCCAGGGCTTA	CAATAACCTGATCTGTGCACCAGTA	XM_415247

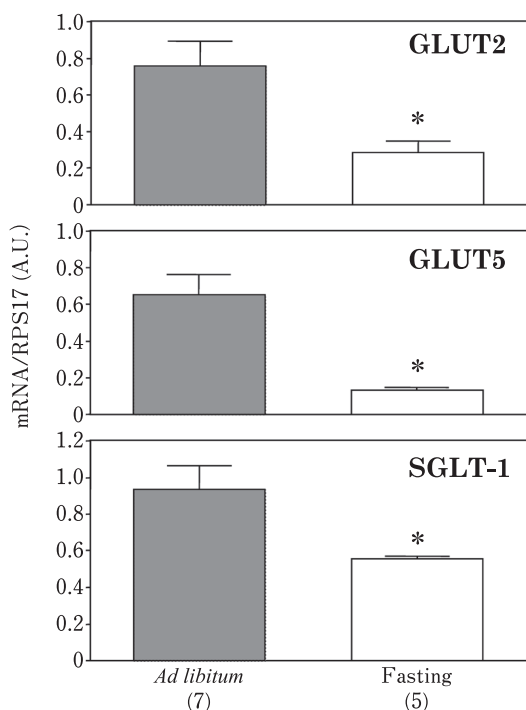RPS17, ribosomal protein S17; GLUT, glucose transporter; SGLT, sodium-dependent glucose transporter.

Fig. 2. **Gene expression of transporters in the intestine of chicks as determined by quantitative RT-PCR.** The values represent the mean±SEM of the number of chicks in parentheses. *$P<0.05$, compared to *ad libitum* group.

four; at 30 min, four; at 60 min, six; fructose (24-h fasting) at 15 min, five; glucose (*ad libitum*) at 15 min, six.

Statistical Analysis

The data were analyzed using StatView software (Version 5, SAS Institute, Cary, NC, USA). All data were evaluated using the Student *t* test. Statistical significance was set at $P<0.05$. Data were expressed as the means±SEM.

Results

Gene Expression of Fructose Transporters

Figure 2 shows the gene expression of GLUT2, GLUT5, and SGLT-1 in the intestine of chicks under *ad libitum* or fasting conditions. The gene expression levels of all transporters in the fasted chicks were lower than those in the *ad libitum* chicks ($P<0.05$). However, the mRNA expression of GLUT2 and SGLT-1 decreased to approximately 40 and 60%, respectively, by 24-h fasting, whereas the fasting-induced decrease in gene expression of GLUT5 was 20% of that of the *ad libitum* condition.

Fructose Uptake Determined by Everted Sac Method

Transport of fructose across the everted intestine sac into the serosal space is shown in Fig. 3. Fructose uptake rapidly increased within 15 min after incubation, and then gradually increased until 60 min. At 15 min after incubation, fructose uptake of the everted intestine in *ad libitum* chicks was approximately two-fold that in the fasting chicks ($P<0.05$), while glucose uptake was less than half that in fasting chicks

($P<0.05$).

Discussion

Starvation reduces sugar absorption in the small intestine because there is no luminal signal to preserve a large amount of transporters in mammals (Ferraris and Carey, 2000). This finding is in good agreement with those of the present study, which showed that the gene expression of fructose-related transporters (GLUT2, GLUT5) and SGLT-1 were reduced by fasting (Fig. 2). Enhancing GLUT5 mRNA expression tends to co-occur with increased expression of GLUT2 and SGLT-1 mRNA (Kishi *et al*., 1999), but the fasting-induced decrease of GLUT5 mRNA expression (Fig. 2, middle panel) was less than that of GLUT2 and SGLT-1 (Fig. 2, upper and lower panels), suggesting that the regulation of GLUT5 is different from the other transporters. Luminal glucose increases intracellular cyclic AMP levels, suggesting the regulation of SGLT-1 in rats (Dyer *et al*., 2003), whereas the regulation of GLUT5 is involved in the adenylate cyclase/PKA pathways (Gouyon *et al*., 2003). Unlike SGLT-1 and GLUT2, GLUT5 rapidly responds to changes in dietary or luminal fructose signaling (Ferraris, 2001). Additionally, GLUT5, but not SGLT-1, mRNA expression in the small intestine is affected by plasma hormones such as glucocorticoids in various species, including chicken (Monterio *et al*., 1999; Douard *et al*., 2008; Ebrahimi *et al*., 2015).

As in mammals, luminal glucose can cross the apical and basolateral membranes in chickens via SGLT-1 and GLUT2, respectively (Ferrer *et al*., 1994; Garriga *et al*., 1997). Additionally, Garriga *et al*. (2004) revealed that GLUT5 is responsible for the apical transport of fructose using the brush-border membrane vesicle of the chicken jejunum. Our result using the everted sac system showed that fructose uptake increased logarithmically with increasing incubation time (Fig. 3). The uptake at 15 min incubation was approximately 60% of that after 60 min incubation, and a similar time course for fructose uptake was observed in the brush-border membrane vesicle test (Garriga *et al*., 2004). Although both the gene expression of GLUT5 and uptake of fructose were decreased by fasting treatment, the ratios of these decreases were not parallel to each other (Figs. 2 and 3). There are two possible explanations for this discordance. One explanation is that the level of mRNA expression cannot entirely parallel the protein level. In a previous study, despite a significant increase in GLUT5 mRNA expression, the number of GLUT5 transporters was not affected (García-Barrios *et al*., 2010). Another explanation is that GLUT2 conducts fructose transport at the apical membrane because GLUT2 appears to be present at the border membrane vesicle of the intestine in rats (Kellett and Helliwell, 2000; Kellett, 2001). Further studies are needed to investigate the time course of mRNA expression and protein for sugar transporters, which depend on the nutritional condition in chicks. Such investigations may be useful for controlling poultry feed and nutrient absorption.

The uptake of glucose in the chick jejunum is approximately two-fold greater than that of fructose at 15 min in-
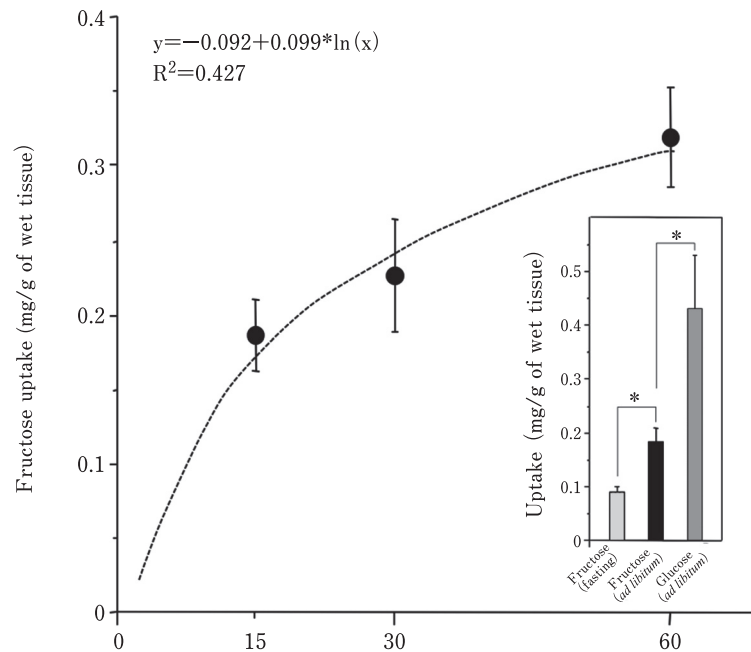
Fig. 3. **Transport of fructose or glucose across everted intestine sac into the serosal space.** The number of chicks in each group was as follows: fructose (*ad libitum*) at 15 min, four; at 30 min, four; at 60 min, six; fructose (fasting) at 15 min, five; glucose (*ad libitum*) at 15 min, six, respectively. The data represent the means±SEM.

cubation (Fig. 3). This finding is in accordance with those of earlier reports, suggesting lower absorption rates of fructose in the small intestine in mammals (Bjorkman *et al.*, 1984; Holloway and Parsons, 1984; Riby *et al.*, 1993). This lower rate of fructose absorption occurs because luminal fructose is transported by facilitated diffusion depending on the concentration gradient of GLUT5, whereas glucose is actively transported by SGLT-1 (Garriga *et al.*, 2004). In fact, SGLT-1, but not GLUT5, can transport glucose via the apical membrane even if the level of luminal glucose is lower than that in the blood (Kellett, 2001).

In this study, we evaluated monosaccharide uptake using the jejunum of chicks (4 days old). This uptake may increase with age because morphological development of the intestine rapidly occurs from day 2 after hatching, with crypt depth increasing until day 7, whereas the villus size continued to increase through day 14 in the jejunum (Uni *et al.*, 1998). Further studies are needed to investigate the uptake or responsiveness of transporters with age. Such investigations may have implications for the growth and health in chickens.

In conclusion, our data revealed that fructose can be absorbed through the jejunum of chickens, with less absorption observed after fasting because of downregulation of related transporter genes in the small intestine of broiler chicks.

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