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Feeding Rats Dietary Resistant Starch Shifts the Peak of SGLT1 Gene Expression and Histone H3 Acetylation on the Gene from the Upper Jejunum toward the Ileum.

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Sodium glucose cotransporter 1 (SGLT1) participates in the incorporation of glucose from the lumen to enterocytes in the small intestine. We examined whether dietary resistant starch (RS), an autoclaved high amylose starch that is digested more slowly than regular cornstarch in the small intestine, alters SGLT1 mRNA levels along the jejunum-ileum of rats. The SGLT1 mRNA level was lower in the upper jejunum in rats fed an RS diet than in those fed a regular cornstarch diet, whereas it was higher in the lower jejunum/upper ileum. Furthermore, using chromatin immunoprecipitation (ChIP) assay, we demonstrated that histone H3 acetylation on the promoter/enhancer and transcriptional regions was reduced in the upper jejunum and elevated in the lower jejunum/upper ileum by feeding rats an RS diet. On the other hand, HNF-1 binding on the region around transcription start site of the SGLT1 gene was not altered in each jejunoileal segment by feeding rats an RS diet. Our results suggest that a shift of the expressional peak of the SGLT1 gene from the upper jejunum toward the ileum by dietary RS is associated with a change of histone H3 acetylation rather than that of HNF-1 binding on the gene.

KEYWORDS: Resistant starch; SGLT1; histone acetylation; chromatin; small intestine

INTRODUCTION

Studies have demonstrated that delaying digestion/absorption of carbohydrates in the small intestine has various beneficial effects for preventing/improving lifestyle-related diseases such as obesity, diabetes, and their complications (*1*, *2*). This is partially because of decreased postprandial hyperglycemia and an improved bacterial environment in the intestine. Indeed, several drugs and food components capable of slowing carbohydrate digestion/absorption are now used in patients with diabetes and obesity. These normally act as inhibitors of disaccharidases (e.g., maltase and sucrase-isomaltase (SI)), which participate in the final digestion of starch hydrolysates and sucrose, or as inhibitors of sodium glucose cotransporter 1 (SGLT1), which is a glucose and galactose transporter expressed in the luminal plasmalemma of enterocytes. Some studies have shown that various starch products such as resistant starch (RS) and high-amylose starch, which are digested more slowly in the small intestine than regular cornstarch, also have these beneficial effects (*1*). An earlier study of ours demonstrated that feeding rats a diet containing high-amylose cornstarch for 14 days led to a decrease in disaccharidases activities in the upper jejunum and an increase in activities in the lower jejunum/upper ileum (*3*). In addition, treating streptozotocin-induced diabetic rats with acarbose, an inhibitor for disaccharidases, for 12 days led to reduced activity and protein levels of sucrase in the jejunum (*4*). Furthermore, many studies have shown that dietary carbohydrate induces expression of SI and SGLT1 genes in the jejunum of rodents (*5*−*7*). These observations suggest that feeding diets with drug/food components capable of slowing carbohydrate digestion/absorption may lead to a shift in the activities of glucose transport, as well as disaccharidases from the upper jejunum toward the ileum along the small intestinal axis. Presumably, this would be through changing the expression of SGLT1 and disaccharidases genes. It is thought that changing these gene expressions as well as their activities from the upper jejunum toward the ileum leads to reduced postprandial hyperglycemia. This is because these proteins expressed in the small intestine, particularly in the upper jejunum, are carbohydrate absorption rate-controlling factors, and lower activities in the upper jejunum lead to delayed carbohydrate absorption. However, it is unclear whether the gene expression of SGLT1 as well as disaccharidases along the jejunum-ileum is altered by feeding such drug/food ingredients.

Studies have indicated that gene expression at the transcriptional level is regulated by transcriptional factors that bind directly to cis-elements located on the upstream region of the genes. Indeed, HNF-1, a nuclear transcriptional factor, has been shown to regulate circadian rhythmical changes of the SGLT1 gene expression in rat jejunum (*8*). However, it remains unclear whether the expressional changes of the SGLT1 gene from feeding rats dietary carbohydrate including RS are indeed regulated by HNF-1. Other studies indicate that histone modifications are important for expressional changes of genes involved in many

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Table 1. Diet Composition

	control	50% RS (g/100 g)	100% RS
Cornstarch	55.0	27.5	–
Hi-maize[a]	–	27.5	55.0
Casein	20.0	20.0	20.0
Lard	10.0	10.0	10.0
Corn oil	5.0	5.0	5.0
Cellulose	5.0	5.0	5.0
AIN93 mineral mixture	3.5	3.5	3.5
AIN93 vitamin mixture	1.0	1.0	1.0
L-cystine	0.3	0.3	0.3
Choline bitartrate	0.2	0.2	0.2

[a] Hi-maize contained more than 60% resistant starch.

aspects of biological processes. Multiple lysine or arginine residues in core histones, particularly H3 and H4, are subjected to posttranslational modifications, including methylation and acetylation; many of these modifications are associated with distinct transcription states (*9*, *10*). Among several identified histone modifications, acetylations of histone H3 are the most extensively studied because regulation of histone H3 acetylation is closely related to the ON/OFF switch of transcription. Indeed, several studies have confirmed that hyperacetylation of histone H3 is associated with the euchromatin region on the genome (*10*−*12*). Our recent studies demonstrated that a diet containing high-carbohydrate induced gene expressions of SGLT1 and SI with concomitant enhancement of acetylation of histone H3 (*13*, *14*). Furthermore, we have recently shown that the induction of SI gene expression by feeding mice a high-carbohydrate diet is closely associated with histone H3 acetylation on the gene rather than the bindings of their nuclear transcriptional factors such as Cdx-2 and HNF-1 (*13*). Therefore, it is likely that intake of drug/food ingredients capable of slowing digestion/absorption of carbohydrate induces a shift of SGLT1 gene expression from the upper jejunum toward the ileum along the small intestinal axis, presumably through a change in histone H3 acetylation on the gene.

In this study, we examined whether feeding rats dietary RS induces the shift of gene expression of SGLT1 from the upper jejunum toward the ileum along the jejunum-ileum. We also investigated whether the gene expressional change along the jejunum-ileum is associated with changes in histone H3 acetylation and HNF-1 binding on the gene.

MATERIALS AND METHODS

Animals. Seven-week-old male Wistar rats (Japan SLC, Shizuoka, Japan) were divided into three groups, control, 50% RS and 100% RS. These received a diet containing regular cornstarch (control diet), a diet in which 50% of the cornstarch in the control diet was replaced with the same amount of RS (50% RS diet), and a diet in which 100% of the cornstarch in the control diet was replaced with the same amount of RS (100% RS diet), respectively. Cornstarch and RS consist of [> 99% carbohydrate (approximately 25% amylose), < 0.5% protein and 0% fat], and [> 99% carbohydrate (approximately 70% amylose), < 0.5% protein and 0% fat], respectively. RS was supplied as hi-maize (Hi-maize1043, Nippon NSC Ltd., Tokyo, Japan), which is made from natural high-amylose maize starch as described previously (*15*) and is classified as type 2 RS. Hi-maize1043 contains more than 60% RS. Details of the diet compositions are shown in **Table 1**. Animals were allowed free access to the diets and water for 7 days. At the end of feeding for 7 days, a 6 h-fasting blood sample was obtained from the tail vein to determine plasma glucose and triacylglycerol concentrations. Rats were then killed by decapitation and the entire small intestine was collected. The experimental procedures used in the present study conformed to the guidelines of the Animal Usage Committee of the University of Shizuoka.

Preparation of Intestinal Samples. The entire jejunoileum was removed and divided into two equal length segments. The upper half was regarded as jejunum and the lower half as ileum. To examine details of the distribution of SGLT1 mRNA along the jejunum-ileum, we further divided the jejunum or the ileum into four equal segments. The four jejunal or ileal segments were numbered beginning at the proximal end of the jejunum or ileum; jejunal (J) segments, J1−4; Ileal (I) segments, I1−4. Each segment was flushed twice with ice-cold 0.9% NaCl solution. A 1-cm segment (100 mg each) was excised from the middle region of each segment and immediately used for RNA extraction. The remaining part of each segment was used for chromatin immunoprecipitation (ChIP) assays.

Real-Time RT-PCR. Intestinal segments for RNA extraction were homogenized by a solution for homogenizing for total RNA extraction (4 M guanidine thiocyanate, 25 mM sodium citrate pH 7.5, 0.5% sarcosyl, 0.1 M 2-mercaptoethanol). The homogenates of the small intestine were immediately frozen using liquid nitrogen and stored at −80 °C until RNA extraction. Total RNA was extracted by the acidified guanidine thiocyanate method, as described by Chomczynski and Sacchi (*16*). Total RNA samples (2.5 μg) were converted into cDNA by reverse transcription using Super Script III Reverse transcriptase (Invitrogen, Tokyo, Japan) according to the manufacturer's instructions. To quantitatively estimate the mRNA levels of SGLT1 and TATA box binding protein (TBP), polymerase chain reaction (PCR) amplification was performed on a Light-Cycler480 instrument system (Roche, Tokyo, Japan). Real-time RT-PCR reactions were carried out in a total volume of 10 μL containing 400 nM each of gene specific primers, cDNA, and SYBR Premix Ex Taq (Takara, Shiga, Japan). Sequences of the PCR primer pairs and the fragment size are shown in **Table 2**. The cycle threshold (CT) values of each gene detected by real-time RT-PCR were converted into signal intensities by the delta−delta method (*17*), which calculates the difference of one CT value as a 2-fold difference between the signal for each gene and the signal for a gene for normalization (TBP). The formula used was $[2^{(\text{CT of SGLT1} - \text{CT of TBP})}]$.

Chromatin Immunoprecipitation (ChIP) Assay. Mucosa removed from the remaining jejunoileal parts were immediately added to ice-cold fixation solution (1% formaldehyde, 4.5 mM Hepes pH 8.0, 9 mM NaCl, 0.09 mM EDTA, 0.04 mM EGTA) in PBS. They were then incubated for 30 min at 37 °C. The reaction was terminated by the addition of glycine to a final concentration of 150 mM. After being washed in FACS solution (1 × PBS (−), 2% bovine serum, 0.05% NaN_3), the samples were sonicated in SDS lysis buffer (50 mM Tris-HCl pH 8.0, 10 mM EDTA pH 8.0, 1% SDS, 0.5 mM PMSF); the DNA size of samples was 200−500 bp. ChIP assays were performed using 2 μg each of specific antibodies, that is, antiacetyl-histone H3 (Milipore, Tokyo, Japan), HNF-1 (Santa Cruz Biotechnology, CA) or control rabbit IgG as previously described (*13*). Precipitated DNA was subjected to real-time RT-PCR using primers corresponding to the indicated regions (**Table 2**). The CT-value of ChIP and input signals detected by real-time RT-PCR were converted to signal intensities using the delta−delta method (*17*). All ChIP signals were normalized to the corresponding input signals. The formula used was $100 \times [2^{(\text{CT IP sample} - \text{CT Input})}]$.

Other Assays. Body weight was measured once every 2 or 3 days. Plasma glucose and triacylglycerol levels were enzymatically determined with commercial kits (Glucose CII-Test Wako and Triglyceride E-Test Wako, Wako Pure Chemical Industries, Osaka, Japan).

Statistical Analysis. Each value was expressed as the mean ± SEM. Significance between two groups was evaluated by Student's *t*-test. $P < 0.05$ was considered to indicate statistical significance. All statistical analyses were performed by Ekuseru-Toukei, an Excel-based statistical software and provided by Social survey Research Information Co. Ltd. (Tokyo, Japan).

RESULTS

Effects of Dietary RS on Body Weight, and Plasma Levels of Glucose and Triacylglycerol of Rats. No differences in body weight or plasma glucose level were seen among rats fed control, 50% RS or 100% RS diets, whereas animals fed 50 or 100% RS showed significantly lower plasma triacylglycerol levels (0.73-fold, $p < 0.05$ and 0.65-fold, $p < 0.01$, respectively) than those fed a control diet. Total starch intake for 7 days was not significant different between control and 100% RS groups (control group, 59.2 ± 2.2 g; 100% RS group, 54.0 ± 2.1 g,), while total fecal weight of

Table 2. Sequences of Oligonucleotide Primers Used

	fragment size	sequence
SGLT1 mRNA	132	5′-TGTACCCTGTGTGGCT-3′ 5′-CGTGCCGCAGTGTTTC-3′
TBP mRNA	146	5′-CCCACAACTCTTCCATTCTCA-3′ 5′-TTTGGAGCTGTGGTACAATCC-3′

region on the SGLT1 gene[a]		fragment size	sequence
SGLT1	−5197 ∼ −5177	90	5′-AGCTGCTTAGAACGGTGTTCA-3′
−5000	−5129 ∼ −5108		5′-GGCAACTGGAAAGTCTGTTAGG-3′
SGLT1	−2218 ∼ −2198	95	5′-TTTCTGTCTGAAGGAGCTTGG-3′
−2000	−2144 ∼ −2124		5′-GCTTCTGTTTTCACGACCATC-3′
SGLT1	−1033 ∼ −1013	87	5′-GGTTTGATGAGCTCCTTGACA-3′
−1000	−967 ∼ −947		5′-CAGTAAGACCCCGAATCAAGA-3′
SGLT1	−482 ∼ −461	123	5′-TCTCCTTACAGTGGTGGGAATC-3′
−500	−380 ∼ −360		5′-AGGAGGATGAGATTGCTGGAT-3′
SGLT1	−59 ∼ −39	84	5′-CTGCTCACCGAACAGACTTTA-3′
+0	3 ∼ 24		5′-AGGTGTGCAGTGCTCTAAGTCA-3′
SGLT1	1268 ∼ 1288	108	5′-TTGCTCTCCTGTGGTTGAACT-3′
+1000	1355 ∼ 1375		5′-AGGGGATCGGAAGCATAGTTA-3′
SGLT1	2063 ∼ 2083	131	5′-AGGGATGGATACAATGGCTTC-3′
+2000	2173 ∼ 2193		5′-GAGTGTTGTTGGAATGGATGC-3′
SGLT1	5173 ∼ 5193	97	5′-CCAACATGACCTGTGGCTTAT-3′
+5000	5249 ∼ 5269		5′-CGCTTAGTCATCGAGGAAATG-3′
SGLT1	10903 ∼ 10924	92	5′-TACTTCTCGAAGGAGTCCAAGC-3′
+10000	10974 ∼ 10994		5′-CTGATGTCATCTCAGCAGCAA-3′
Sucrase-iso	88102 ∼ 88125	103	5′-CTGCTTCCTCTTCTACCACAGTCC-3′
maltase	88185 ∼ 88204		5′-CAGAGACCCACAGCCAGACA-3′
(SI) Over+2000[b]			

[a] The region is denoted relative to the transcription initiation site. [b] The primer is used in place of that of a region far downstream of the SGLT1 gene (2000 bp downstream of the transcription termination site).

rats fed a 100% RS diet was 2.7-fold greater than those fed a control diet (control group, 10.7 ± 0.5 g; 100% RS group, 28.6 ± 3.1 g, $p < 0.01$). It was estimated that digested RS was approximately 52%, as calculated by the formula [(Total starch intake of rats fed RS) − (Total fecal weight of rats fed RS)]/[(Total starch intake of rats fed regular cornstarch) − (Total fecal weight of rats fed regular cornstarch)] × 100.

Distribution of SGLT1 Gene Expression along the Jejunum-Ileum of Rats Fed a Control or RS Diet. To examine whether dietary RS alters SGLT1 gene expression along jejunum-ileum, we determined SGLT1 mRNA levels in eight jejunoileal segments of rats fed either control (regular cornstarch) or RS diets for 7 days by real-time RT-PCR assay. As shown in **Figure 1**, rats fed a control diet had a peak of SGLT1 mRNA at the most proximal part of the jejunum (J1) and the levels decreased gradually toward the distal ileum, while those fed the 100% RS diet had a peak of SGLT1 mRNA at the most distal part of jejunum (J4) and the levels gradually decreased away from the J4 to the distal ileum. SGLT1 mRNA levels in the proximal jejunum (J1 and J2) were 0.56- and 0.76-fold, respectively, lower in the 100% RS group than in controls ($p < 0.05$). By contrast, SGLT1 mRNA levels in the distal jejunum (J4) and proximal ileum (I1) were 1.5- and 2.1-fold, respectively, higher in the 100% RS group than in controls (J4, $p < 0.05$; I1, $p < 0.01$). The 50% RS group tended to reduce SGLT1 gene expression in the upper jejunum (J1 and J2) and induce gene expression in the lower jejunum (J4) and upper ileum (I1 and I2), compared with the control group. Significant differences were observed in I1 ($p < 0.05$). Jonckheere-Terpstra Test showed the reduction of SGLT1 mRNA in J1 ($p = 0.0049$) and J2 ($p = 0.0212$), and the induction of the mRNA in J4 ($p = 0.0111$), I1 ($p = 0.002$) and I2 ($p = 0.002$) were dose dependent.

Effects of Dietary RS on Acetylation of Histone H3 Bound to the SGLT1 Gene along the Jejunum-Ileum of Rats. To examine whether histone H3 acetylation on the SGLT1 gene is connected with the change of SGLT1 mRNA by feeding rats an RS diet, we performed ChIP assay using an antibody for acetylated histone H3 in samples of each jejunoileal segment of rats fed

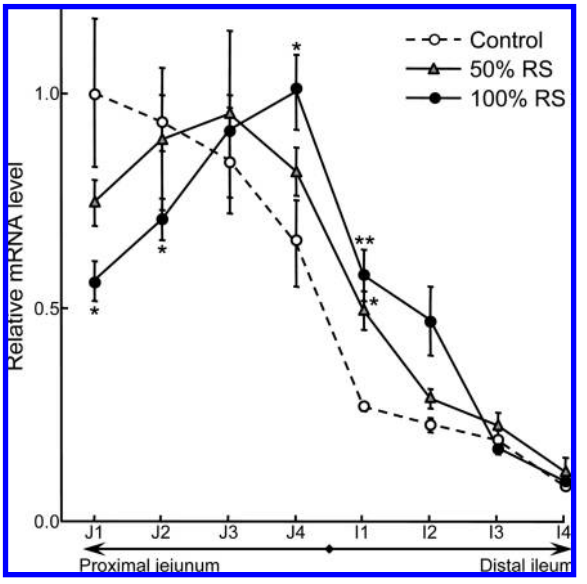

Figure 1. Distribution of SGLT1 gene expression along the jejunoileum of rats fed a control or RS diet. Wistar rats were fed control (regular cornstarch), 50% resistant starch (RS) or 100% RS diet for 7 days. Total RNA extracted from jejunoileum divided into equal one-eighths was quantified by real-time RT-PCR with SGLT1 gene-specific primer. Messenger RNA levels were normalized to the levels of TBP mRNA. Means ± SEM of 5−6 animals are shown. Asterisks indicate significant differences, in the same jejunoileal segment, compared with rats fed a control diet by Student's *t* test (*$p < 0.05$, **$p < 0.01$).

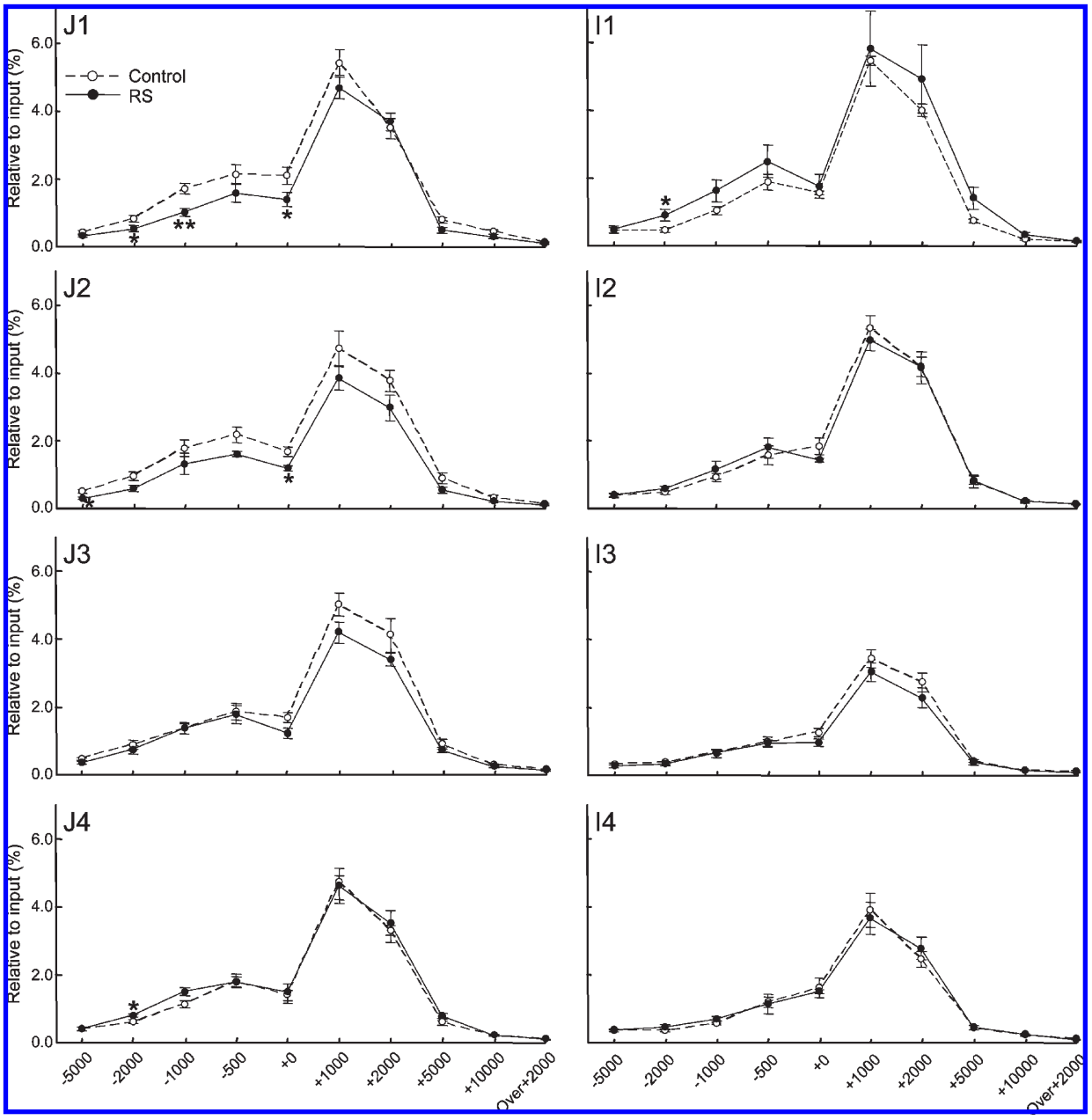

Figure 2. Effects of dietary resistant starch on acetylation of histone H3 bound to the SGLT1 gene in various regions along the jejunoileum of rats. Wistar rats were fed control (regular cornstarch) or resistant starch (RS) diet for 7 days. Genomic DNA, cross-linked to nuclear proteins in the jejunoileum divided into equal one-eighth segments, was sonicated and precipitated by the antibodies against acetylated histone H3. ChIP signals were detected by quantitative real-time RT-PCR and normalized to input signals. Means ± SEM of 5−6 animals are shown. Asterisks indicate significant differences, on the same region of the SGLT1 gene, compared with rats fed a control diet by Student's *t* test (*$p < 0.05$, **$p < 0.01$).

control or 100% RS diets. We selected several regions of the SGLT1 gene, such as regions upstream (−5000, −2000, −1000 and −500) and transcription (+0, +1000, +2000, +5000 and +10000). We also selected a region far downstream of the SI gene (+2000 bp downstream of the transcription termination site) as an unrelated region for a negative control, because the downstream region of the SGLT1 gene was not clearly identified in rat. ChIP signals for IgG were <0.028% per input. None of the ChIP signals of acetylated histone H3 in the region far downstream of the SI gene differed from that of IgG (data not shown). On the other hand, ChIP signals of acetylated histone H3 in the regions of the promoter/enhancer (−5000, −2000, and −1000 bp) and transcription (+0, +5000, and +10000 bp) of the SGLT1 gene in J1 of the 100% RS group tended to be lower than those of the controls ($p < 0.094$). Significant differences were observed in the regions of the promoter/enhancer (−2000 and −1000 bp) and transcription (+0 bp) (region −2000 and +0 bp, $p < 0.05$; region −1000 bp, $p < 0.01$) (**Figure 2**). Similarly, signals in the regions of the promoter/enhancer (−5000, −2000, and −500 bp) and transcription (+0 and +5000 bp) in J2 and of the transcription (+0 bp) in J3 of the 100% RS group tended to be lower than those of the controls ($p < 0.093$). There were significant differences in the regions of the promoter/enhancer (−5000 bp) and transcription (+0 bp) in J2 ($p < 0.05$). By contrast, signals in the regions of the promoter/enhancer (−2000 and −1000 bp) in J4 and the promoter/enhancer (−2000 bp) in I1 tended to be higher in the 100% RS group than those in the controls ($p < 0.06$). Significant differences between the two groups were observed in the region of the promoter/enhancer (−2000 bp) in J4 and I1 ($p < 0.05$).

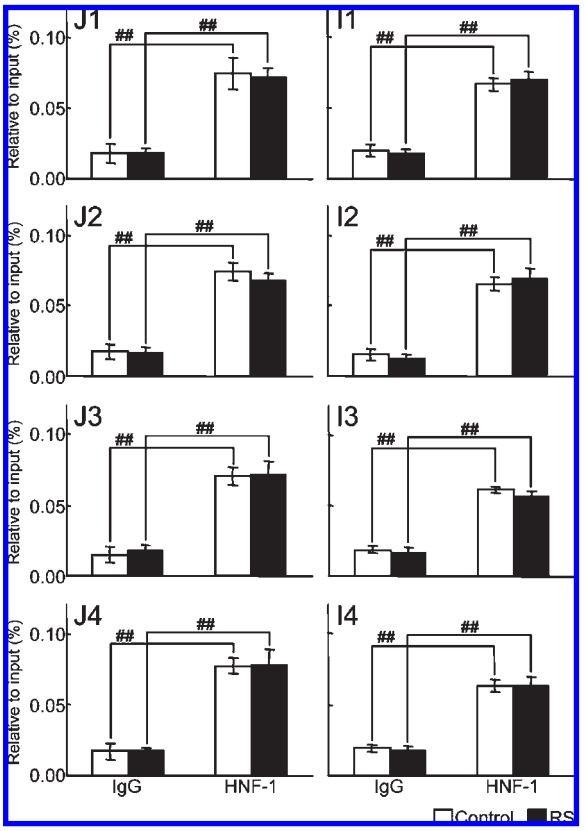

Figure 3. Effects of dietary resistant starch on HNF-1 binding to the SGLT1 gene at the transcriptional start site along the jejunoileum of rats. Wistar rats were fed control (regular cornstarch) or resistant starch (RS) diet for 7 days. Genomic DNA, cross-linked to nuclear proteins in the jejunoileum divided into equal one-eighth segments, was sonicated and precipitated by antibodies against HNF-1 or IgG (control). ChIP signals of HNF-1 or IgG in each jejunoileum segment were detected by quantitative real-time RT-PCR and normalized to input signals. Means ± SEM of 5−6 animals are shown. Sharps indicate significant differences in each jejunoileal segment compared with IgG signals for the same group by Student's *t*-test. (##$p < 0.01$).

Effects of Dietary RS on HNF-1 Binding to the Region around the Transcriptional Initiation Site of the SGLT1 Gene along the Jejunum-Ileum of Rats. To examine whether HNF-1 binding on the cis-element of the SGLT1 gene is involved in the change of SGLT1 mRNA in rats fed a 100% RS diet, we performed ChIP assays using an antibody for HNF-1 in samples of each jejunoileal segment. We selected the region around the transcriptional start site of SGLT1 (+0 bp) since it has been reported that a putative cis-element of HNF-1 is located around the transcriptional start site of the SGLT1 gene in rat (*8*). As shown in **Figure 3**, ChIP signals by HNF-1 were detected in the region around the transcriptional start site on the SGLT1 gene in each jejunoileal segment (J1-I4) of both the control and 100% RS groups. These were approximately 3-fold higher than those by IgG in each jejunoileal segment of each group ($p < 0.01$); whereas ChIP signals by HNF-1 in each jejunoileal segment were not significantly different between the two groups.

DISCUSSION

We have reported that feeding rats a high-amylose cornstarch diet leads to a shift of the peak of disaccharidases activities from the upper jejunum toward the ileum (*3*). In this study, we showed that feeding dietary RS shifted the peak of SGLT1 gene expression from the upper jejunum toward the ileum in rats (**Figure 1**). These findings indicated that feeding rats a diet that includes slowly digested starch induces the shift of the gene expressional peak of SGLT1 and the activities of disaccharidases from the upper jejunum toward the ileum. Several studies have reported that expressions of the SGLT1 and SI genes are coordinately enhanced by dietary starch and sucrose (*5*−*7*). Thus, it is very likely that the shift of the gene expressional peak of the SGLT1 gene by dietary RS would be regulated at a transcriptional level.

Studies have shown that acetylation of histone H3 as well as the binding of transcriptional factors on genes is important for regulating transcriptional activation (*10*−*12*). Our earlier studies demonstrated that acetylation of histone H3 on the SGLT1 and SI genes and their gene expressions were enhanced by a high carbohydrate diet (*13*, *18*). In this study, we demonstrated that consumption of RS reduced the levels of histone H3 acetylation in the upper jejunum in the regions of both the promoter/enhancer (−5000, −2000, and −1000 bp) and transcription (+0 bp) regions of the SGLT1 gene (**Figure 2**; J1 and J2), while it increased the levels of histone H3 acetylation in the region of the promoter/enhancer (−2000 bp) of the SGLT1 gene in the lower jejunum/upper ileum (**Figure 2**; J4 and I1). These results suggested that the shift of the SGLT1 gene expression peak from the upper jejunum toward the ileum by feeding dietary RS is associated with a change in histone H3 acetylation on the SGLT1 gene. By contrast, the binding of HNF-1, which is known as a crucial transcriptional factor for the SGLT1 gene, on the gene, was observed around the cis-element located on the transcription initiation site (approximately 3-fold higher than the IgG signals). However, the HNF-1 binding along the jejunum-ileum axis was not altered by feeding rats an RS diet (**Figure 3**). ChIP signals in the transcriptional region (+0) of the acetylated histone H3 were > 16-fold higher than those of HNF-1. In general, the amount of histones is more abundant in genome than those of transcription factors including HNF-1. This is because histones bind all genome and participate in all genome folding, whereas nuclear transcriptional factors including HNF-1 are for the transcriptional regulation of specific genes through their binding to specific DNA sequences located on the genes. Thus, it is reasonable that the ChIP signals of acetylated histone H3 were much higher than those of HNF-1. Several earlier studies have shown that regular cornstarch intake as well as force-feeding of fructose in mice induces jejunal expressions of SGLT1 and SI genes and histone acetylations on the genes (*13*, *14*, *18*). Furthermore, the SI induction by regular cornstarch intake was more strongly associated with increased acetylation levels of histones than with increased binding of nuclear transcriptional factors such as Cdx-2 and HNF-1 on the gene (*13*). Taken together, these reports suggest that by feeding rats an RS diet a change in histone H3 acetylation rather than a change in HNF-1 binding leads to the shift of the expression peak of the SGLT1 gene along the jejunoileal axis through enhancing recruitment of transcriptional and mRNA elongational complexes on the SGLT1 gene.

Studies have demonstrated that proteins with bromodomains, which are known to bind to acetylated histones, have a role in the regulation of transcription of genes on acetylated histones. Bromodomain proteins with histone acetyltransferase (HAT) activity such as CBP/p300, PCAF and GCN5 induce acetylations of histones on the gene; furthermore, bromodomain proteins such as TAFII250 and Brg1, which are known as proteins in transcriptional complexes, recruit mRNA transcriptional complexes in the promoter/enhancer region and mRNA elongational complexes in the transcriptional region (*19*−*21*). Our results in this study suggested that changing histone H3 acetylation from the upper jejunum toward the ileum on the SGLT1 gene by dietary RS should be affected by the recruitment of transcriptional

Table 3. Effects of Dietary RS on Body Weight and Plasma Levels of Glucose and Triacylglycerol of Rats[a]

	at start			at 7 days		
	control	50% RS	100% RS	control	50% RS	100% RS
Body weight (g)	153.8 ± 4.5	154.7 ± 5.8	154.1 ± 4.2	188.0 ± 4.0	186.0 ± 6.4	181.8 ± 2.9
Plasma glucose (mg/dL)	−;	−;	−;	114.5 ± 2.4	119.9 ± 3.4	115.9 ± 2.4
Plasma triacylglycerol (mg/dL)	−;	−;	−;	31.6 ± 2.5	23.0 ± 0.3[b]	20.5 ± 0.6[c]

[a] Values are expressed as means ± SEM for 5−6 animals. [b] $p < 0.05$, [c] $p < 0.01$; significantly different from control (Student's t test).

complexes, elongational complexes, and HATs in the promoter/enhancer and transcriptional regions of the SGLT1 gene. Further studies should investigate whether the recruitment of proteins with HATs and transcriptional/elongational complexes on the SGLT1 gene is associated with the alteration of gene expression and histone H3 acetylation.

It remains unknown which signals alter histone H3 acetylation on the SGLT1 gene as well as its gene expression along the jejunum-ileum in rats fed the RS diet. It seems likely that the amount of carbohydrates available for glucose absorption that flow into each jejunoileal segment would be involved in eliciting the signal. It is reported that a diet containing an inhibitor of disaccharidases, acrabose, repressed glucose uptake in the proximal small intestine (*22*). Additionally, greater amounts of carbohydrate are present in the ileum of rats fed a diet containing RS than those fed a diet without RS (*23*). Thus, the intake of the diet containing RS possibly leads to decreasing available carbohydrate levels in the upper small intestine and increasing the levels in the lower small intestine, compared with a diet containing regular starch. We have already shown that histone H3 acetylation on the SI and SGLT1 genes, as well as their gene expressions, are enhanced in the jejunum of mice fed a high carbohydrate diet (*13*, *14*). Taken together, it is likely that the glucose available for uptake by the absorptive cells in each jejunoileal segment would be attributable to the induction of histone H3 acetylation on the SGLT1 and SI genes as well as their gene expressions. Although the physiological relevance of the RS diet-induced change in distribution of SGLT1 gene transcripts along the jejunoileal axis needs to be determined, we have observed in this study that feeding a diet containing RS in the rats reduced plasma triacylglycerol levels (**Table 3**). Furthermore, feeding a diet containing RS in type 2 diabetic Goto-Kakizaki rats for 3 months led not only to the shifting of the peak of SGLT1 mRNA levels from the upper jejunum toward the ileum, but also to amelioration of the diabetic condition (manuscript in preparation). One of the reasons for preventing diabetes by using dietary RS may be that the shift of SGLT1 mRNA caused by the change of histone H3 acetylation on the gene from the upper jejunum toward the ileum leads to a decrease of postprandial hyperglycemia and a decrease of plasma triacylglycerol following a decrease of postprandial hyperglycemia. However, there are many possible reasons for improvements in diabetes caused by RS intake. RS intake decreases postprandial hyperglycemia even if SGLT1 expression along the jejunum-ileum is unchanged, since RS itself is slowly digested in the small intestine. RS intake also reduces total energy intake and improves the bacteria environment. Further studies should investigate whether the shift of SGLT1 mRNA from the upper jejunum to the ileum caused by the change of histone H3 acetylation on the SGLT1 gene reduces postprandial hyperglycemia in diabetic animals.

In conclusion, we demonstrated that dietary RS shifts the peak of histone H3 acetylation on the SGLT1 gene, as well as its gene expression, along the jejunoileal axis from the upper jejunum toward the ileum in rats.

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