Dietary Resistant Starch Reduces Levels of Glucose-Dependent Insulinotropic Polypeptide mRNA along the Jejunum-Ileum in Both Normal and Type 2 Diabetic Rats

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It has been reported that the circulating glucose-dependent insulinotropic polypeptide (GIP) levels were reduced by an intake of some foods/drugs capable of delaying carbohydrate digestion/absorption. In this study, we revealed that feeding rats with dietary resistant starch reduced the GIP mRNA levels along the entire length of the jejunoileum in both Wistar and type 2 diabetic GK rats.

Key words: resistant starch; GIP; jejunum; ileum; GK rats

Glucose-dependent insulinotropic polypeptide (GIP) is synthesized in and secreted from K cells which are located at high density in the proximal small intestine and gradually decrease in density toward distal small intestine.1,2) Many previous studies have demonstrated that the circulating GIP levels were elevated by carbohydrate and fat intake, and that the predominant action of GIP was to augment glucose-stimulated insulin secretion from the islet β cell.3) Excessive GIP release by overeating/speed-eating of carbohydrates induces greater insulin secretion and could lead to the onset/progression of life-style related diseases such as diabetes and obesity by abnormal glucose and lipid metabolism. Indeed, some studies have observed that the circulating GIP levels were higher in obese and diabetic subjects than in healthy subjects.4–7) It is thus believed that repressing the excessive GIP and insulin secretion caused by delaying the digestion/absorption of carbohydrates is important for preventing/improving such diseases. Several human studies have already shown that the postprandial induction of circulating GIP was repressed by a simultaneous intake of acarbose,8–10) an inhibitor of disaccharidases in the small intestine, and a soluble fiber guar gum11) and resistant starch (RS),12) which consists of an autoclaved high-amylose starch, is known to undergo digestion slowly and has the characteristics of a dietary fiber. It seems likely that the foods and drugs capable of delaying carbohydrate digestion alleviate excessive GIP secretion. Although many studies have reported that GIP secretion into the blood was regulated by nutrient factors, there is only limited information about whether dietary factors regulate GIP gene expression in the small intestine; Tseng et al.13) have found that the levels of GIP mRNA in the rat duodenum were enhanced within a few hours after glucose ingestion.13) However, it is still unclear whether delaying carbohydrate digestion/absorption in the gastrointestinal tract leads to an expressional change of the GIP mRNA level in the small intestine. We examined in this study whether feeding dietary RS would alter the GIP mRNA level along the jejunoileum of Wistar and type 2 diabetic Goto-Kakizaki (GK) rats.

Male seven-week-old Wistar and twelve-week-old GK rats (Japan SLC, Hamamatsu, Japan) were used in this study. The Wistar rats were allowed free access to diets with three levels of RS (0% (control), 50% or 100%) for 1 week, and the GK rats were given the diets with two levels of RS (control or 100%) for 12 weeks. The diabetic animals of both the control and 100% RS groups were pair-fed during the experimental period. Details of the diet compositions are shown in Table 1. RS was supplied as Hi-maize1043 (Nippon NSC Ltd., Tokyo, Japan) which is made from natural high-amylose maize starch as described previously14) and contains more than 60% RS. It was confirmed that the food intake during the experimental period and the body weight at the end of feeding were not significantly different among the groups in both experiments, although the body weight tended to be lower in the RS-fed groups than in the control group for the Wistar (control group, 188 ± 3.1 g; 50% RS group, 184 ± 5.3 g; 100% RS group, 180 ± 3.2 g) and the GK (control group, 363.8 ± 14.1 g; 100% RS group, 323.8 ± 3.7 g) rats.

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At the end of the feeding period, the animals were fasted for 6 h, killed at 10:00–11:00 by decapitation, and the complete jejunoileum was excised. The experimental procedures used in the present study conformed with the guidelines of the Animal Usage Committee of the University of Shizuoka. The jejunoileum of the Wistar rats was divided into eight segments of equal length (segments 1–4, jejunum; segments 5–8, ileum), while that of the GK rats was divided into four equal lengths (segments 1–2, jejunum; segments 3–4, ileum). The segments were flushed twice with an ice-cold 0.9% NaCl solution. A 1-cm segment (100 mg each) was excised from the middle region of the small intestinal segment, and immediately used for RNA extraction. Total RNA was extracted by the acidified guanidine thiocyanate method, as described by Chomczynski and Sacchi. The total RNA samples (2.5 μg) were converted into cDNA by reverse transcription with SuperScript III Reverse transcriptase (Invitrogen, Japan) according to the manufacturer’s instructions. To quantitatively estimate the GIP mRNA levels, polymerase chain reaction (PCR) amplification was performed with a LightCycler 480 instrument (Roche, Japan). Real-time 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, Japan). The GIP mRNA levels were quantified by real-time PCR and normalized to those of TBP mRNA. Each value is the mean ± SE for five animals per group. Asterisks indicate significant differences compared with the levels in the control rats (⁎P < 0.05, ⁎⁎P < 0.01).

![Fig. 1. Distribution of GIP mRNA along the Jejunum-Ileum Axis of Normal Rats Fed on Diets with Three Levels of Resistant Starch for 1 Week.](image1)

Wistar rats were fed on diets with three levels of resistant starch (0% (control), 50% or 100%) for 1 week, and total RNA was extracted from the jejunoileum which was divided into eight segments (segments 1–4, jejunum; segments 5–8, ileum). The GIP mRNA levels were quantified by real-time PCR and normalized to those of TBP mRNA. Each value is the mean ± SE for five animals per group. Asterisks indicate significant differences compared with the levels in the control rats (⁎P < 0.05, ⁎⁎P < 0.01).

![Fig. 2. Distribution of GIP mRNA along the Jejunum-Ileum Axis of Type 2 Diabetic Rats Fed on Diets with Two Levels of Resistant Starch for 12 Weeks.](image2)

GK rats were fed on diets with two levels of resistant starch (0% (control) or 100%) for 12 weeks, and total RNA was extracted from the jejunoileum which was divided into four segments (segments 1–2, jejunum; segments 3–4, ileum). The GIP mRNA levels were quantified by real-time PCR and normalized to those of TBP mRNA. Each value is the mean ± SE for six animals per group. Asterisks indicate significant differences compared with the levels in the control rats (⁎⁎P < 0.01).
control group. As shown in Fig. 2, the GIP mRNA levels throughout the entire jejunileum were also lower in the 100% RS group of the GK rats than in the control group. The GIP mRNA levels in segments 1, 2, 3 and 4 were 0.35-, 0.48-, 0.37- and 0.22-fold lower, respectively (p < 0.01), in the 100% RS group than in the control group. It has already been shown that GIP secretion was repressed by dietary RS, supplementation of dietary fiber and α-glucosidase inhibitors. Additionally, the circulating GIP levels were lower in humans that had ingested starch than in those that had ingested glucose. Taking our results and these earlier reports together, the delay in glucose absorption caused by starch being degraded slowly in the lumen should lead to a reduction in GIP gene expression. It is still unknown which signal altered the GIP mRNA levels in the rats fed the diet with RS. It should be noted that the GIP mRNA levels in the distal segments of the rats were also reduced by dietary RS in our study, in spite of the results of a study showing that the carbohydrate contents in the ileum and cecum were much higher in rats after an RS intake than in those after a sugar intake. This indicates that the GIP mRNA levels along the jejunum-ileum axis were not correlated with the glucose contents in each part of the jejunileum. Thus, some factors changed by delayed carbohydrate digestion/absorption such as insulin, free fatty acid as well as glucose may affect GIP gene expression along the jejunum-ileum axis. Further studies should be undertaken to identify which factors alter the intestinal expression of the GIP gene along the jejunum-ileum axis.

In contrast to the GIP mRNA levels, the plasma GIP levels at the end of feeding were unchanged between the control and RS groups in both the experiments on the normal (control group, 3.70 ± 0.64 ng/ml; 50% RS group, 3.79 ± 0.44 ng/ml; 100% RS group, 3.56 ± 0.15 ng/ml) and diabetic (control group, 2.57 ± 0.21 ng/ml; 100% RS group, 2.69 ± 0.22 ng/ml) rats. The repression of the GIP mRNA levels along the jejunumileum by RS ingestion may lead to reducing postprandial GIP release into the blood. Further studies are needed to determine the GIP protein levels a few hours after carbohydrate intake in the small intestine and in the blood of rats with lower GIP mRNA levels caused by RS ingestion.

RS is known to be a beneficial food for preventing/improving diabetes by alleviating postprandial hyperglycemia and hyperalimentation. We found in this study that a dietary RS intake not only repressed the GIP mRNA levels in the small intestine but also alleviated the levels of fasting plasma glucose (control group, 156.9 ± 2.4 mg/100 ml vs. 100% RS group, 124.3 ± 4.1 mg/100 ml, p < 0.01) and triacylglycerol (control group, 84.1 ± 12.2 mg/100 ml vs. 100% RS group, 41.3 ± 10.1 mg/100 ml, p < 0.05) in the diabetic rats. Some recent studies have reported that the circulating GIP concentrations were associated with the development of diabetes in humans. Although it is assumed that there are many reasons for the improvement of diabetes by the RS intake, and it needs to be investigated whether the repression of excessive GIP secretion can lead to the prevention/improvement of diabetes and the associated complications, the results of our current study support this notion.

In conclusion, we have demonstrated that feeding dietary RS reduced the levels of GIP mRNA along the entire jejunileum in both Wistar and type 2 diabetic GK rats.

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References


