

## Article

# Acute Low-intensity Treadmill Running Induces Intestinal Glucose Transporters via GLP-2 in Mice

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**Abstract:** Exercise affects various organs. However, its effects on nutrient digestion and absorption in the intestinal tract are not well understood. A few studies have reported that exercise training increases the expression of carbohydrate digestion and absorption molecules. Exercise was also shown to increase the concentration of blood glucagon like peptide-2 (GLP-2), which regulates carbohydrate digestion and absorption in small intestinal epithelium. Therefore, we investigated the effects of exercise on intestinal digestion and absorption molecules and the levels of GLP-2. 6-wk-old of male mice were divided into 2 groups; sedentary (SED) and low-intensity exercise (LEx). LEx mice were required to run on a treadmill (12.5 m/min, 60 min), whereas SED mice rested. All mice were euthanized 1 h after exercise or rest and plasma, jejunum, ileum, and colon were sampled. Samples were analyzed using EIA and immunoblotting. The levels of plasma GLP-2 and the expression of the GLP-2 receptor, sucrase-isomaltase (SI), and glucose transporter (GLUT2) in the jejunum were increased in LEx group. We showed that acute low-intensity exercise affects the intestinal carbohydrate digestion and absorption molecules via GLP-2. Our results suggest that exercise might provide new benefits to the small intestine for people with intestinal frailty.

**Keywords:** low intensity exercise; intestine; sodium-dependent glucose transporter; glucose transporter 2; glucagon like peptide 2

## 1. Introduction

Exercise has been shown to affect the body by causing various physiological and biochemical changes. In particular, exercise therapy has been tried out for the management of dementia, weak locomotion-associated sarcopenia, and diabetes [1-6]. Several studies have demonstrated the effects of exercise on various organs, and recently, effects have also been reported on the small intestine [7,8]. These effects on the small intestine were shown to mainly target intestinal permeability and inflammation [9-11], and less the digestive and absorptive capability. In order for the host organism to absorb nutrients from food, the process of digestion and absorption needs to occur in the small intestine. However, after clinical treatment, such as fasting and total parenteral nutrition (TPN),

the digestive and absorptive capability of the small intestine has been shown to be remarkably reduced and associated with a decrease in the expression of nutrient digestion- and absorption-related genes [12, 13]. To date, 2 studies have reported the effects of exercise on intestinal glucose transporters. Motiani et al. reported that 2 wk of moderate intensity training increased the expression level of glucose transporter 2 (GLUT2) in the intestine of mice [14]. In addition, Kondo et al. reported that 6 wk of swimming exercise induced substantial adaptations in the digestive capability of the pancreas and absorptive capability in the small intestine in rats [15]. Therefore, exercise might be effective in improving the digestive and absorptive molecules of carbohydrates in the small intestine. Nevertheless, the mechanism underlying this adaptation remained unknown.

The expressions of carbohydrate transporters and saccharidases in the small intestine are known to be regulated by nutritional stimulation. The sodium-dependent glucose transporter (SGLT1) and GLUT2 typical glucose transporters, as well as the sucrase-isomaltase (SI) known as typical disaccharidase, are known to react sensitively to dietary saccharides, resulting in their increased expression. It was recently found that glucagon-like peptide-2 (GLP-2) were also increased the expression of these proteins [16-19]. In particular, GLP-2 is produced from endocrine L cells in the ileum and colon as proglucagon [20,21] and several studies on its effects in humans and animal models have suggested it as a therapeutic agent for intestinal weaknesses, such as TPN, short bowel syndrome, and inflammatory bowel disease [22-27]. Among them, Drucker et al. have demonstrated the efficacy of GLP-2 analogs in human [23], which is currently pharmaceutical approved as *Teduglutide* in the USA. In contrast, acute aerobic exercise has been reported to increase the plasma levels of GLP-1 [28], which is a hormone produced from the same proglucagon and secreted by the same L cells as GLP-2. Therefore, it is conceivable that exercise might increase the plasma levels of GLP-2, leading to increased expression of carbohydrate transporters and disaccharidases in the small intestine. Investigation of the exercise-induced increase in the digestion and absorption of intestinal carbohydrates and elucidation of its mechanism would provide new insights regarding the implementation of exercise therapy in conditions of decreased digestion and absorption.

In this study, we hypothesized that exercise increases the expression levels of carbohydrate digestion- and absorption-related proteins via GLP-2 and tested this hypothesis using an acute low-intensity exercise model in mice.

## 2. Materials and Methods

### 2.1. Animal treatment and acute exercise

The present study was conducted in accordance with the principles and guidelines for international animal care and was approved by the Animal Experimental Committee, University of Tsukuba (approval number: 20-492). Briefly, 6-wk-old ICR male mice were purchased from CLEA Japan Inc. (Tokyo, Japan). Mice were bred and maintained in an air-conditioned animal house under specific pathogen-free conditions and subjected to 12/12 h light and dark cycles. Animals were fed standard mice pellet and water ad libitum. At 7 wk of age, all mice were familiarized with treadmill running for 20–30 min. Mice were divided into the sedentary (SED; n = 6) and low-intensity exercise (LEx; n = 6) groups and were allowed to rest for 24 h. The LEx group performed acute treadmill running for 1 h at a rate of 12.5 m/min. Mice were subjected to fasting for 2 h before exercise. After acute exercise, mice were allowed to rest for 1 h and then euthanized. Mice in the SED group were sacrificed after 2 h of fasting followed by a rest period.

## 2.2. Tissue Sampling

Mice were sacrificed by cervical dislocation under anesthesia. Blood samples were collected into EDTA-2Na-containing 1.5 mL tubes and then centrifuged at 5,000 rpm and 4 °C for 10 min to obtain plasma. The small intestine was removed, and duodenum was isolated and flushed using ice-cold phosphate buffered saline (PBS [2.7 mM KCl, 1.76 mM KH<sub>2</sub>PO<sub>4</sub>, 137 mM NaCl, 10 mM Na<sub>2</sub>HPO<sub>4</sub>]). Subsequently, the small intestine was cut in half and the duodenum side of the small intestine was used as the jejunum, whereas the remaining side was used as the ileum. Approximately 1 cm of the central part of the jejunum and ileum were used for morphological analysis. After that, the colon was collected, and approximately 1 cm of the central part of the colon was used for morphological analysis. The remaining samples were used for immunoblotting analyses. Samples were frozen in liquid nitrogen and stored at -80 °C until further analysis.

## 2.3. Histochemical staining and immunohistochemical analysis

Tissues were fixed in 10 % formalin phosphate buffer (Nacalai Tesque, Kyoto, Japan) for at least 24 h. Fixed ileum and colon samples were immersed in 70 % ethanol. Samples were then paraffin embedded, sectioned (4 µm thick), and stained with hematoxylin and eosin (H&E) (MUTO PURE CHEMICALS CO., LTD, Tokyo, Japan).

Hydrated samples were autoclaved (120 °C, 10 min) in 10 mM citrate buffer (pH 6.0) for antigen activation before immunohistochemical (IHC) analysis. Subsequently, samples were permeabilized using 0.3 % H<sub>2</sub>O<sub>2</sub> in methanol and blocked with 5 % goat serum (Sigma-Aldrich, St. Louis, MO, USA) in PBS for 1 h at 20~25 °C. GLP-2 antibody (1:100, A5009, Abclonal Technology, Woburn, MA, USA) was diluted in 10 % goat serum in PBS and incubated with samples overnight at 4 °C. Samples were washed thrice with PBS and incubated with secondary antibody (1:150, #7074, horseradish peroxidase-conjugated anti-rabbit IgG, Cell Signaling Technology, Danvers, MA, USA) for 30 min at 25 °C. Finally, samples were washed thrice with PBS, and stained using the DAB stain kit (Nacalai Tesque, Kyoto, Japan). All images were captured using a microscope (BZ-X710, Keyence, Osaka, Japan).

## 2.4. Measurements of blood lactate and plasma GLP-2, glucose, triglycerides (TG), and nonesterified fatty acids (NEFA)

Blood lactate was measured before and after exercise using Lactate Pro2 (LT-1730, ARKRAY, Inc., Kyoto, Japan). Plasma GLP-2 was measured using a mouse GLP-2 EIA kit (Yanaihara Institute Inc., Shizuoka, Japan). Plasma glucose, TG, and NEFA were measured using Glucose C II-test Wako and lab assay kits (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan). All assays were performed according to the manufacturer's instructions.

## 2.5. Immunoblotting

Total proteins were extracted from the jejunum using RIPA buffer (1 M Tris-HCl, 5 M NaCl, 0.5 M EDTA, 0.5 % sodium dodecyl sulfate, 2.5 % sodium deoxycholate, 5 % NP-40, and distilled water including proteinase inhibitor [cOmplete™ mini, Roche, Basel, Switzerland] and phosphatase inhibitor [PhosSTOP™, Roche, Basel, Switzerland]) tablets. Lysates were centrifuged at 15,000 g for 15 min at 4 °C. The concentration of total

protein for each sample was measured using a BCA protein assay kit (Takara Bio, Shiga, Japan). Subsequently, 10  $\mu$ g of total protein per lane was used for gradient gel electrophoresis. Proteins were transferred to polyvinylidene fluoride (PVDF) membranes, which were then incubated with primary antibodies against SGLT1 (1:1,000, A11976, ABclonal Technology, MA, Woburn, USA), GLUT2 (1:1,000, 20436-1-AP, Proteintech Group Inc., Rosemont, IL, USA), GLUT5 (1:1,000, 27571-1-AP, Proteintech Group Inc., Rosemont, IL, USA), GLP-2R (1:1,000, A6602, ABclonal Technology, Woburn, MA, USA) and Sucrase-Isomaltase (1:1,000, sc-393470, SantaCruz Biotechnology, Dallas, TX, USA). Horseradish peroxidase-conjugated anti-rabbit IgG (1:5,000, #7074, Cell Signaling Technology, MA, Danvers, USA) and anti-mouse IgG (1:5,000, #7076, Cell Signaling Technology, MA, Danvers, USA) were used as secondary antibodies. Signals were detected using a chemiluminescence reagent (ECL Prime Western Blotting Detection Reagent, GE Healthcare, Chicago, IL, USA). Blots were scanned using a chemiluminescence imaging system (FUSION FX7.EDGE, Vilber Lourmat, Marne-la-Vallee, France).

## 2.6. Statistical analysis

Data are shown as the mean  $\pm$  standard deviation (SD). Data were subjected to unpaired *t*-test to evaluate statistical significance. A value of  $p < 0.05$  was considered statistically significant. Statistical analyses were performed using GraphPad Prism 8.4.3 (GraphPad Software, San Diego, CA, USA) for Mac.

## 3. Results

### 3.1. Acute exercise did not affect blood lactate

As the low-intensity exercise was considered to be acute exercise in this study, we measured the levels of blood lactate to validate the intensity of the exercise. We did not observe any change in the concentrations of blood lactate (Figure 1).

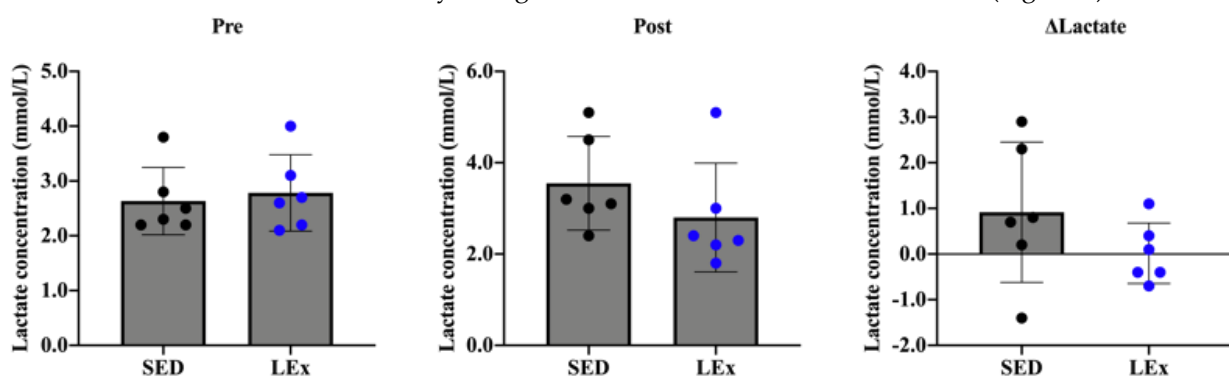


Figure 1. Effects of acute exercise on the concentrations of blood lactate. The concentrations of blood lactate were measured before and after exercise.  $\Delta$ Lactate indicates the amount of change in blood lactate before and after exercise. SED; sedentary group ( $n = 6$ ). LEx; low-intensity exercise group ( $n = 6$ ).

### 3.2. Acute exercise induced the levels of GLP-2 in the plasma and the expression of GLP-2R in jejunum

We measured the GLP-2 in the ileum, colon and plasma using immunohistochemical analysis and EIA. In addition, we measured the expression of the GLP-2 receptor in

jejunal samples. We found that GLP-2 in both ileum and colon (Figure 2-A). In particular, we observed that the levels of GLP-2 increased in the LEx group (Figure 2-B). In addition, the expression of GLP-2R was also demonstrated to be increased in LEx group (Figure 2-C).

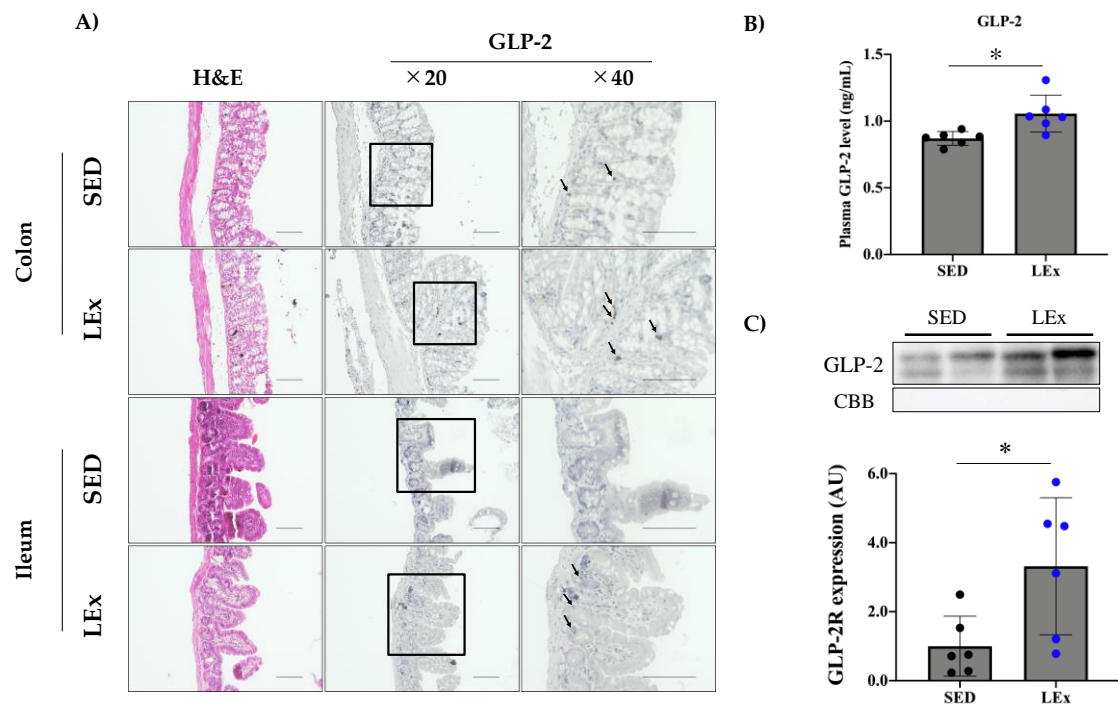


Figure 2. Effects of acute exercise on GLP-2 in the ileum, colon, and plasma. A) Hematoxylin&eosin stain and immunohistochemical (IHC) staining for GLP-2. Scale bar, 100  $\mu$ m. Black arrows indicate GLP-2. B) Levels of plasma GLP-2. C) Expression levels of GLP-2R in jejunum. GLP-2; glucagon-like peptide-2, GLP-2R; glucagon-like peptide-2 receptor. CBB; Coomassie Brilliant Blue stain. To verify equal protein loading across lanes, membranes were stained with CBB. The CBB bands represent the 35-48 kDa region where many internal controls are present. SED; sedentary group (n = 6). LEx; low-intensity exercise group (n = 6). \*p < 0.05.

### 3.3. Acute exercise did not affect the concentrations of plasma glucose, TG, and NEFA.

To investigate the effect of acute exercise on blood parameters, we measured the levels of plasma glucose, TG, and NEFA. Accordingly, we did not observe any change in any of these blood parameters (Figure 3).

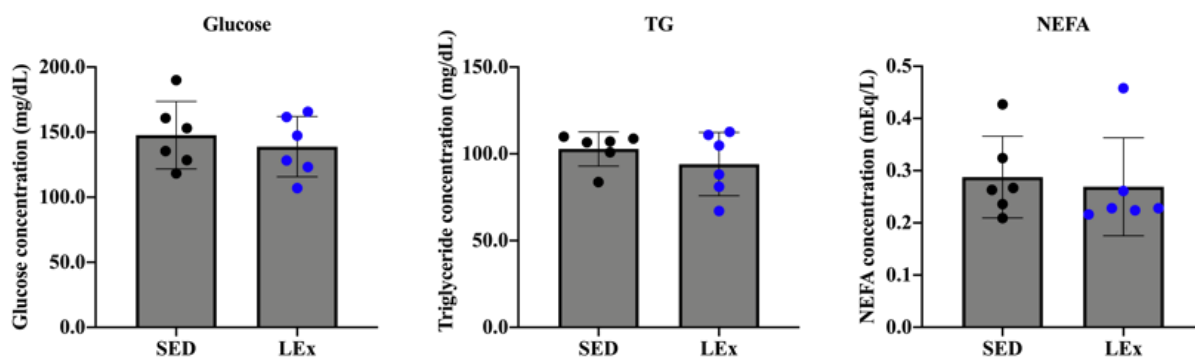


Figure 3. Effects of acute exercise on the concentrations of plasma glucose, TG, and NEFA. TG; triglycerides, NEFA; nonesterified fatty acids, SED; sedentary group (n = 6). LEx; low-intensity exercise group (n = 6).



### 3.4. Acute exercise induced the protein expression levels of the SI and GLUT2 in jejunum.

To investigate the effect of acute exercise on the digestion and absorption of carbohydrates, we measured the expression of typical carbohydrate transporters and disaccharidases. We found that the expression of both SI and GLUT2 increased in the LEx group (Figure 4).

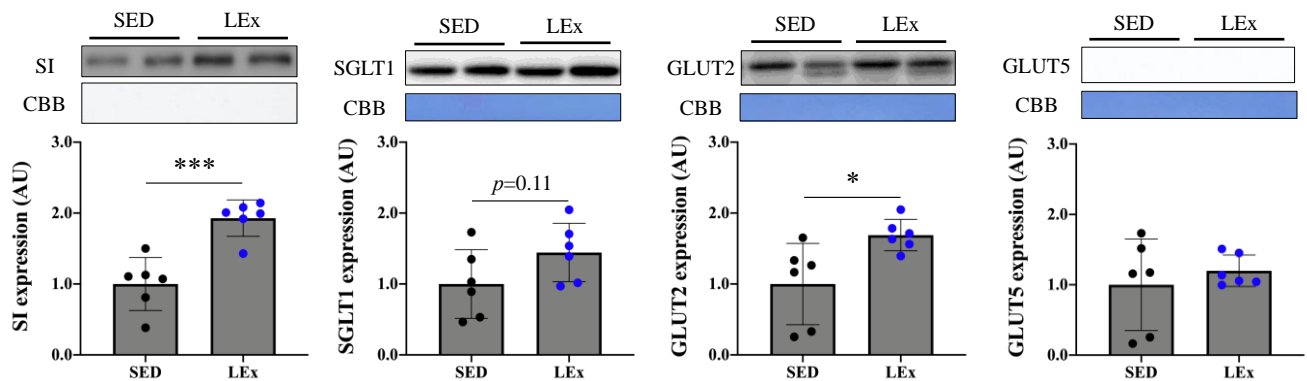


Figure 4. Effects of acute exercise on carbohydrate absorption- and digestion-related proteins. \*\*\*p < 0.0005, \*p < 0.05. SI; sucrase-isomaltase. SGLT1; Na<sup>+</sup>-dependent glucose transporter 1. GLUT2; glucose transporter 2. GLUT5; glucose transporter 5. CBB; Coomassie brilliant blue stain. To verify equal protein loading across lanes, membranes were stained with CBB. The CBB bands represent the 35-48 kDa region where many internal controls are present. SED; sedentary group (n = 6). LEx; low-intensity exercise group (n = 6).

## 4. Discussion

This study was conducted with a focus on the potential alterations in the levels of GLP-2 and carbohydrate digestion/absorption related molecules in the intestine following acute exercise. Exercise already has been known to enhance the tight junctions in the intestine [10], but its effect on the digestive and absorptive capability of the intestine is less well-known. To test this, we subjected mice to a regimen of acute exercise and examined the changes in the expression of disaccharidase and monosaccharide transporters, as well as in the GLP-2 positive regulator, in the intestine.

We did not detect any changes in the concentrations of blood lactate between pre- and post-exercise ( $\Delta$ Lactate). Changes in the concentrations of blood lactate are known to be dependent on the intensity of the exercise. Therefore, we correctly assumed that the exercise intensity used in this study was low intensity.

We hypothesized that exercise induces the levels of GLP-2. We accordingly found that the plasma levels of GLP-2 were increased in the LEx group (Fig. 2-B). Only one other study has investigated the changes in the levels of GLP-2 after exercise. Janssen-Duijghuijsen et al. reported that the serum levels of GLP-2 were increased in well-trained healthy male cyclists after two exercise sessions [29]. However, their protocol included the intake of casein protein and a multisugar solution. As the secretion of GLP-2 is known to be increased by nutrient stimulation and changes in blood biochemical parameters, such as TG and NEFA [30], the levels of GLP-2 could have been affected by the intake of casein protein and multisugar solution. In contrast, in our study, mice were not fed after exercise and no differences were observed in the levels of TG or NEFA (Fig. 3). Therefore, to the best of our knowledge, this is the first study reporting an increase in the levels of GLP-2 due to low-intensity exercise alone.

The expression of the GLP-2 receptor in the jejunum was also increased in parallel with the increase in the level of GLP-2. A number of carbohydrate digestion and absorption factors are known to be downstream targets of the GLP-2 signaling via GLP-2R. In particular, we found that the protein expression levels of SI and GLUT2 were increased, while SGLT1 also showed an increasing trend, although not significant (Fig. 4). More specifically, GLP-2 has been reported to upregulate the gene expression of SI, which is known to be decreased by TPN [18]. Au et al. reported that the jejunal expression of GLUT2 was promoted by GLP-2 in rats [16]. In addition, Cheeseman et al. found that administration of GLP-2 increased the glucose uptake and expression of SGLT1 in the rat jejunum [19]. Therefore, we assumed that the exercise-induced trend toward increased GLUT2 and SGLT1 in this study was induced via GLP-2 signaling. It has been reported that 6 wk of swimming training increased the expression of SGLT1, which was explained as an adaptation of the intestine to long-term exercise [15]. As the exercise style of this study was treadmill running, whereas that of the previous study was swimming, it was not possible to make a general comparison; however, GLP-2 might be a key factor in the adaptation of the intestine to exercise.

There was one limitation in this experiment: we were not able to measure the active form of GLP-2. There are two types of GLP-2 in blood, active (GLP-2<sup>1-33</sup>) and inactive (GLP-2<sup>3-33</sup>), and what we measured was the total amount of both in this study. Therefore, we will be able to examine in detail how effective exercise is in inducing GLP-2 by measuring the active form of GLP-2. In addition, experiments of long-term training by treadmill running would help to clarify the relationship between GLP-2 and exercise.

To date, the effects of exercise on the small intestine have mainly focused on tight junctions. In this study, we reported the effects of exercise on the digestion and absorption of carbohydrates, which constitute one of the most essential nutrient sources for humans. As the intestine is atrophied in TPN and short bowel syndrome, the restoration of its functions are important. In this regard, the digestion and absorption of nutrients are important, and exercise might contribute to the recovery through the increased levels of GLP-2. In the future, using TPN and short bowel syndrome models, would better clarify the effects of exercise on intestinal functions.

## 5. Conclusions

This study examined the effects of acute low-intensity exercise on the levels of GLP-2 and intestinal digestion and absorption of carbohydrates molecules. Exercise increased the secretion levels of GLP-2 and consequently the expression of SI and GLUT2. Hence, exercise might likely improve the digestion and absorption of carbohydrates in the small intestine via the elevated levels of GLP-2.

**Author Contributions:** methodology, K.A., H.F., T.N., K.Y., Y.Y. and T. Sugasawa; validation, K.A., and T. Suzuki; formal analysis, K.A. and T. Suzuki; investigation, K.A., T. Suzuki, N.O., M.Y., S.F., Y.K. and K.T.; writing—original draft preparation, K.A.; writing—review and editing, T. Suzuki, N.O., M.Y., S.F., Y.K., and K.T.; visualization, K.A.; supervision, Y.K., and K.T.; project administration, project administration; funding acquisition, K.T. All authors have read and agreed to the published version of the manuscript.

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**Data Availability Statement:** Data can be made available on request.

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