

Resistant maltodextrin promotes fasting glucagon-like peptide-1 secretion and production together with glucose tolerance in rats

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Abstract

Glucagon-like peptide-1 (GLP-1), which is produced and released from enteroendocrine L cells, plays pivotal roles in postprandial glycaemia. The ingestion of resistant maltodextrin (RMD), a water-soluble non-digestible saccharide, improves the glycaemic response. In the present study, we examined whether the continuous feeding of RMD to rats affected GLP-1 levels and glycaemic control. Male Sprague–Dawley rats (6 weeks of age) were fed an American Institute of Nutrition (AIN)-93G-based diet containing either cellulose (5%) as a control, RMD (2.5 or 5%), or fructo-oligosaccharides (FOS, 2.5 or 5%) for 7 weeks. During the test period, an intraperitoneal glucose tolerance test (IPGTT) was performed after 6 weeks. Fasting GLP-1 levels were significantly higher in the 5% RMD group than in the control group after 6 weeks. The IPGTT results showed that the glycaemic response was lower in the 5% RMD group than in the control group. Lower caecal pH, higher caecal tissue and content weights were observed in the RMD and FOS groups. Proglucagon mRNA levels were increased in the caecum and colon of both RMD and FOS groups, whereas caecal GLP-1 content was increased in the 5% RMD group. In addition, a 1 h RMD exposure induced GLP-1 secretion in an enteroendocrine L-cell model, and single oral administration of RMD increased plasma GLP-1 levels in conscious rats. The present study demonstrates that continuous ingestion of RMD increased GLP-1 secretion and production in normal rats, which could be stimulated by its direct and indirect (enhanced gut fermentation) effects on GLP-1-producing cells, and contribute to improving glucose tolerance.

Key words: Resistant maltodextrin: Fructo-oligosaccharides: Glucagon-like peptide-1: Glucose tolerance: Proglucagon

Glucagon-like peptide-1 (GLP-1) is a gastrointestinal hormone produced and released from enteroendocrine L cells. The primary function of GLP-1 is to enhance glucose-induced insulin secretion (the incretin effect), which maintains normal glucose homeostasis in the postprandial state⁽¹⁾. GLP-1 also has a proliferative effect on pancreatic β -cells, and multiple effects external to the pancreas, including the liver, adipose tissue, cardiovascular system and central nervous system⁽²⁾. Owing to the incretin effect, GLP-1 receptor agonists have been recently used for the treatment of type 2 diabetes^(3,4), together with dipeptidyl peptidase-IV (DPP-IV) inhibitors that have been broadly used for the treatment of inflammatory diseases⁽⁵⁾. Because GLP-1 and another incretin, glucose-dependent insulintropic polypeptide (GIP), are immediately inactivated by degradation with plasma DPP-IV, inhibitors for DPP-IV can help preserve physiological activity of GLP-1 and GIP^(1,5). Increasing endogenous GLP-1 by

possible dietary factors could be effective in improving glucose tolerance and preventing glucose intolerance. Recent studies have shown that the stimulatory effect on GLP-1 by single oral administration of dietary factors such as proteins/peptides^(6–8) and an amino acid^(9,10) effectively lowers the glycaemic response in animal models and/or human subjects.

Resistant maltodextrin (RMD) is a water-soluble, non-viscous and non-digestible saccharide⁽¹¹⁾. Its average molecular weight is approximately 2000 Da. Although RMD reportedly lowers the glycaemic response^(12,13) and postprandial TAG elevation⁽¹⁴⁾, and also promotes mineral absorption⁽¹⁵⁾, the mechanisms involved in the improvement of glucose tolerance are poorly understood.

RMD is fermented by luminal microbiota, resulting in increased proportions of total bacteria and bifidobacteria, and enhanced SCFA production in animals⁽¹⁵⁾ and humans^(11,16).

Abbreviations: DPP-IV, dipeptidyl peptidase-IV; FOS, fructo-oligosaccharides; GIP, glucose-dependent insulintropic polypeptide; GLP-1, glucagon-like peptide-1; GLUTag cells, glucagon-like peptide-1-producing cell line; IPGTT, intraperitoneal glucose tolerance test; RMD, resistant maltodextrin.

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The continuous ingestion of fermentable, non-digestible saccharides such as fructo-oligosaccharides (FOS)^(17,18), inulin^(19,20) and resistant starch⁽²¹⁾ increases GLP-1 levels in animal models and human subjects. The phenomenon is explained by the prebiotic effect of these fermentable saccharides. SCFA produced by the gut microbiota are capable of stimulating GLP-1 secretion, and the G protein-coupled receptor FFAR2 (GPR43) has been shown to function in L cells to sense luminal SCFA, and thereby induce GLP-1 secretion⁽²²⁾. The fact that continuous ingestion of RMD improves glucose tolerance⁽¹²⁾ raises the possibility that GLP-1 secretion and/or production is associated with this effect.

In the present study, we examined whether continuous ingestion of RMD and FOS by rats affected their glucose tolerance, plasma GLP-1 levels and GLP-1 production in the small and large intestines. In addition, the stimulatory effect of single oral administration of RMD, and the direct effects of RMD on GLP-1 secretion were investigated in conscious rats, and in a GLP-1-producing enteroendocrine cell model, respectively.

Materials and methods

Animals and diets

Male Sprague–Dawley rats (5 weeks of age) were purchased from Japan SLC, Inc., and were fed an American Institute of Nutrition (AIN)-93G-based diet⁽²³⁾ (control diet) for a 1-week acclimatisation period. Each rat was housed in a separate cage, and had free access to the diet and water, except for the days preceding glucose tolerance test at week 6 and killing at week 7. The experiment was performed in a temperature-controlled room maintained at $23 \pm 2^\circ\text{C}$ with a 12 h light–12 h dark cycle (08.00–20.00 hours light period). Rats were divided into five groups and were fed the AIN-93G diet-based test diet (Table 1) for 7 weeks. All or half of the cellulose (50 g/kg diet)

in the control diet was replaced with RMD (Fibersol-2; Matsutani Chemical Industry Company) or FOS (Meiologo-P; Meiji Company Limited) to yield final concentrations of 25 g/kg (2.5%) and 50 g/kg (5%) in the individual test diets. The study was approved by the Hokkaido University Animal Committee, and the animals were maintained in accordance with the Hokkaido University guidelines for the care and use of laboratory animals. Body weight and food intake were measured every 2–3 d.

Glucose tolerance test

An intraperitoneal glucose tolerance test (IPGTT) was performed on rats 6 weeks after feeding the test diets. Rats were fasted overnight, and basal (fasting) blood was collected from the tail vein for the measurements of glucose, insulin and total GLP-1 levels. After 15 min, the glucose solution was intraperitoneally injected (1 g/kg) and blood samples were collected from the tail vein at 0, 15, 30, 60, 90 and 120 min after injection. Blood samples were collected into tubes containing heparin (final concentration 50 IU/ml; Ajinomoto Company, Inc.), aprotinin (final concentration 500 Kallikrein inhibitor units (kIU)/ml; Wako Pure Chemical Industries Limited) and DPP-IV inhibitor (final concentration 50 $\mu\text{mol/l}$, DPP4-010; Millipore Corporation). Plasma was separated by centrifugation at 2500 g for 10 min at 4°C and frozen at -80°C until glucose, insulin and GLP-1 measurements were taken. Plasma glucose concentration was measured using the Glucose CII Test Kit (Wako). Plasma insulin concentration was measured using the Rat Insulin ELISA (AKRIN-010T; Shibayagi Company Limited) and Multi Species GLP-1 Total ELISA (EZGLP1T-36K; Millipore), respectively. Homeostatic model assessment for insulin resistance (HOMA-IR)⁽²⁴⁾ was calculated using the following equation:

$$\text{HOMA-IR} = \text{insulin } (\mu\text{U/ml}) \times \text{glucose (mg/dl)} / 405,$$

where 1 mg insulin = 26 IU.

Table 1. Composition of the test diet (g/kg diet)

Ingredients	Control	2.5% RMD	5% RMD	2.5% FOS	5% FOS
Maize starch	397.486	397.486	397.486	397.486	397.486
Casein*	200	200	200	200	200
Dextrinised maize starch†	132	132	132	132	132
Sucrose	100	100	100	100	100
Soyabean oil	70	70	70	70	70
Fibre					
Cellulose‡	50	25	–	25	–
RMD§	–	25	50	–	–
FOS	–	–	–	25	50
Mineral mixture¶	35	35	35	35	35
Vitamin mixture¶	10	10	10	10	10
L-Cys	3	3	3	3	3
Choline bitartrate	2.5	2.5	2.5	2.5	2.5
tert-Butylhydroquinone	0.014	0.014	0.014	0.014	0.014
Energy density (kJ/g)	16.5	16.6	16.7	16.7	16.9

RMD, resistant maltodextrin; FOS, fructo-oligosaccharides; AIN, American Institute of Nutrition.

* Acid Casein (Fonterra Limited).

† TK-16 (Matsutani Chemical Industry Company Limited).

‡ Avicel PH102 (Asahi Kasei Chemicals Corporation).

§ RMD (Fibersol 2; 4.19 kJ/g; Matsutani Chemical Industry).

|| FOS (Meiologo-P; 8.37 kJ/g; Meiji Company Limited).

¶ Mineral and vitamin mixtures were prepared according to the AIN-93G formulation.

Portal blood and tissue collection

After 7 weeks, blood samples were collected from the portal veins of rats under sodium pentobarbital anaesthesia (50 mg/kg body weight, Somnopenyl injection; Kyoritsu Seiyaku Corporation) into a syringe containing heparin (final concentration 50 IU/ml), aprotinin (final concentration 500 kIU/ml; Wako Pure Chemical Industries Limited) and DPP-IV inhibitors (final concentration 50 μ mol/l). Plasma was collected and stored as described above for the measurements of glucose, insulin, GLP-1 (total and active) and GIP. Active GLP-1 and total GIP levels were measured using the GLP-1 (Active) ELISA (EGLP-35K; Millipore) and Rat/Mouse GIP (total) ELISA (EZRMGIP-55K; Millipore), respectively. Active GLP-1 represents intact GLP-1 (GLP-1_{7–36}, GLP-1_{7–37}) that exerts insulin-releasing activity. Total GLP-1 includes both intact and inactivated forms of GLP-1 (GLP-1_{9–36} and GLP-1_{9–37} degraded by DPP-IV), and represents secreted GLP-1.

After the rats were killed by exsanguination, the jejunum, ileum, caecum and colon were isolated. Luminal contents of the jejunum, ileum and colon were washed with cold saline, then mucosa (5 cm segment) and 2 cm segments were collected from the middle region of these tissues for proglucagon mRNA analysis and GLP-1 content measurements, respectively. The tissue weight of the caecum as well as weight and pH of the caecal content were measured. After washing with cold saline, the caecal tissue was separated into halves; one half was collected for GLP-1 measurements and from the other half, the mucosa was scraped for proglucagon mRNA analysis. The intestinal segments were immediately frozen in liquid N₂ and stored at -80°C . Mucosa samples were immediately transferred into tubes containing buffer RLT (RNeasy Mini Kit; Qiagen) and frozen in liquid N₂, and then stored at -80°C . Mesenteric, retroperitoneal and epididymal adipose tissue weights were measured.

Measurement of glucagon-like peptide-1 content in intestinal tissues

Intestinal segments were homogenised in ethanol acid solution (ethanol–12 M-HCl–water 74:25:1; 5 ml/g tissue)⁽²⁵⁾ and extracted for 24 h at 48°C . After centrifugation (2000 g for 20 min), the supernatants were collected and diluted with saline (1000-fold) to measure total GLP-1 levels by ELISA. Tissue protein contents were measured using Lowry's method.

Real-time PCR

Using a real-time PCR system⁽²⁶⁾, mRNA expression levels were determined. Total RNA was extracted using the RNeasy Mini kit (Qiagen), according to the manufacturer's instructions. Complementary DNA was synthesised using the ReverTra Ace qPCR Master Mix with gDNA Remover (Toyobo Company Limited), according to the manufacturer's instructions. Gene expression levels were determined using the Mx3000P Real-Time PCR System (Stratagene) and TaqMan Gene Expression Assay (Life Technologies Company) with rat gene-specific pre-designed TaqMan primers and probe sets

(Rn99999916_s1 for *GAPDH* (glyceraldehyde 3-phosphate dehydrogenase) and Rn00562293_m1 for proglucagon). Relative expression levels were calculated for each sample after normalisation to those of *GAPDH* as a reference gene using the standard curve method.

Glucagon-like peptide-1 secretion study using a glucagon-like peptide-1-producing cell line (GLUTag cells)

GLUTag cells (courtesy of Dr D. J. Drucker, University of Toronto, Toronto, Canada), a murine GLP-1-producing entero-endocrine cell line, were grown in Dulbecco's modified Eagle's medium (catalogue no. 12 100-038; GIBCO), supplemented with 10% fetal bovine serum, 50 IU/ml of penicillin and 500 μ g/ml of streptomycin in a humidified 5% CO₂ atmosphere at 37°C . Cells were routinely trypsinised and subcultured after reaching 80–90% confluency. The GLUTag cells were grown in forty-eight-well culture plates at a density of 1.25×10^5 cells/well for 2 d until they reached 80–90% confluency. Cells were washed twice with HEPES buffer (140 mM-NaCl, 4.5 mM-KCl, 20 mM-HEPES, 1.2 mM-CaCl₂, 1.2 mM-MgCl₂, 10 mM-glucose and 0.1% bovine serum albumin, pH 7.4) to remove the culture media. The cells were then exposed to the test agents dissolved in the same buffer for 60 min at 37°C . Supernatants were collected from the wells, centrifuged at 800 g for 5 min at 4°C to remove the remaining cells, and then stored at -50°C , until GLP-1 concentration was measured using a commercial enzyme immunoassay kit (Yanaihara Institute, Inc.).

Glucagon-like peptide-1 secretory responses after single oral administration of resistant maltodextrin in rats

To examine the acute effect of orally administered RMD on GLP-1 secretion, blood samples were collected from the jugular veins of conscious rats. Male Sprague–Dawley rats (7 weeks of age) equipped with jugular catheters were used in the experiment⁽⁷⁾. After a 3–4 d recovery period, rats were fasted overnight. RMD, maltodextrin (PINEDEX2; Matsutani Chemical Industry) and ZeinH⁽²⁷⁾ were each dissolved in deionised water that was orally administered (2 g/kg), by using a feeding tube (5 Fr; Atom Medical Company). Saline (8 ml/kg) was administered as a control treatment. Blood samples were collected before and after oral administration of the test solutions. Plasma total GLP-1 levels were measured as described above.

Statistical analyses

Data are expressed as means with their standard errors of the mean. Statistical analyses were performed using JMP Pro version 10.0 software (SAS Institute, Inc.). Statistical significance was assessed using one-way (Figs. 1–6) or two-way (Fig. 7) ANOVA. Significant differences ($P < 0.05$) between the mean values were determined using Tukey–Kramer or Dunnett's test, as appropriate.

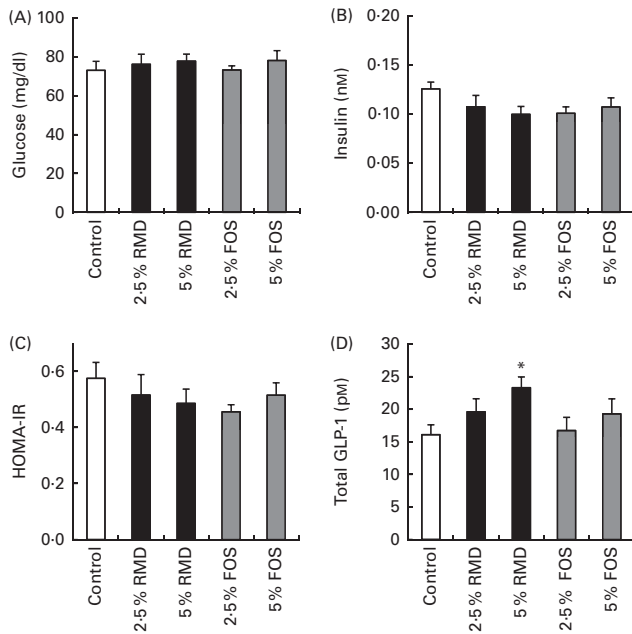


Fig. 1. Fasting glucose (A), insulin (B), glucagon-like peptide-1 (GLP-1) (D) levels and homeostatic model assessment for insulin resistance (HOMA-IR) (C) in the tail vein plasma of rats after 6 weeks of feeding the test diet. Blood samples were collected from the tail vein of rats fasted overnight after 6 weeks of feeding the test diet (15 min before intraperitoneal glucose administration). Glucose, insulin and total GLP-1 levels were measured in the plasma. HOMA-IR was calculated using glucose and insulin concentrations. To convert glucose in mg/dl to mmol/l, multiply by 0.0555. Values are means ($n = 7-8$), with their standard errors represented by vertical bars. *Mean value was significantly different from that of the control group ($P < 0.05$; Dunnett's test). RMD, resistant maltodextrin; FOS, fructo-oligosaccharides.

Results

After 6 weeks of feeding the test diet, rats were fasted overnight and basal glucose (Fig. 1(A)) and insulin (Fig. 1(B)) levels were measured. No significant differences among the treatment groups were observed; however, a slight decline in insulin levels and HOMA-IR (Fig. 1(C)) was observed in the RMD and FOS groups. Plasma total GLP-1 levels (Fig. 1(D)) increased dose-dependently in both the RMD and FOS groups, and the 5% RMD group showed a significantly ($P = 0.036$) higher GLP-1 concentration, compared with the control group ($P < 0.05$; Dunnett's test).

Glucose solution was injected intraperitoneally 15 min after the fasting blood samples were collected. The RMD group showed a dose-dependent reduction in the overall glycaemic response, and glucose level after 15 min was significantly lower in the 5% RMD group than in the control group ($P = 0.047$; Fig. 2(A)). The 2.5% FOS group had a significantly lower glycaemic response after 30 min ($P = 0.035$), compared with the control group. The AUC for changes in glucose concentration was significantly lower ($P = 0.028$) in the 5% RMD group, and the other three groups had lower tendency (as indicated by 'a,b' in the figure), compared with the control group (Fig. 2(B)).

After 7 weeks of feeding the test diet, caecal tissue weights were significantly increased by the RMD (2.5 and 5%, both $P < 0.01$) and FOS (5%, $P < 0.01$) treatments (Table 2).

The weights of caecal content were increased significantly by the RMD treatments compared with the control group ($P = 0.023$ for the 2.5% RMD group and $P = 0.01$ for the 5.0% RMD group, respectively). Conversely, the pH of caecal content was significantly decreased in a dose-dependent manner in rats fed the RMD (2.5 and 5%, both $P < 0.01$) and FOS (5%, $P < 0.01$) diets. The final body weights of the rats and their daily food intake did not differ among the treatment groups. The weights of the mesenteric, retroperitoneal and epididymal adipose tissues were slightly lower in the RMD and FOS groups, but significant difference was not detected. In the early period of the experiment, slightly soft faeces but not diarrhoea were observed in both the RMD and FOS groups.

Fasting portal glucose levels did not differ among the treatment groups (Fig. 3(A)), but insulin levels were significantly lower in the 5% RMD ($P = 0.011$) and 5% FOS ($P = 0.024$) groups (Fig. 3(B)) than in the control group. Portal GLP-1 levels increased dose-dependently in response to the RMD and FOS treatments, and the 5% RMD ($P = 0.007$) and 5% FOS ($P = 0.041$) groups had significantly higher active GLP-1 levels than the control group (Fig. 3(C)). Total GLP-1 level was also significantly higher in the 5% RMD group ($P = 0.006$, Fig. 3(D)). Portal GIP levels were similar in all the groups (Fig. 3(E)).

GLP-1 concentration, corrected according to the total tissue protein concentration (pmol/mg protein), was increased overall, across the intestinal region from the proximal to the distal. GLP-1 concentration was significantly decreased in the jejunum of rats fed the FOS diet, compared with the control group ($P = 0.004$ for the 2.5% FOS group and $P = 0.033$ for the 5.0% FOS group, respectively; Fig. 4(A)), but was not increased by any test diet in the other intestinal regions (Fig. 4(A)–(D)). However, the GLP-1 content in the whole

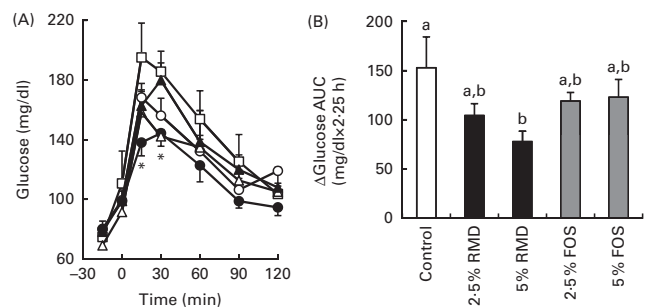


Fig. 2. Plasma glucose levels in rats under the intraperitoneal glucose tolerance test (IPGTT) after 6 weeks. After 6 weeks of feeding the test diet, an IPGTT was performed in rats fasted overnight. Glucose solution was intraperitoneally injected (1 g/kg) at 0 min, and then blood samples were collected from the tail vein. Glucose levels were measured in the plasma (A). The AUC for changes in glucose levels relative to basal levels (B) was calculated using the trapezoidal rule. To convert glucose in mg/dl to mmol/l, multiply by 0.0555. Values are means ($n = 7-8$), with their standard errors represented by vertical bars. *Mean value was significantly different from that of the control group at the same time point ($P < 0.05$; Tukey–Kramer test). ^{a,b}Mean values with unlike letters were significantly different between the treatment groups ($P < 0.05$; Tukey–Kramer test). □, Control; ■, 2.5% resistant maltodextrin (RMD); ●, 5% RMD; △, 2.5% fructo-oligosaccharides (FOS); ▲, 5% FOS.

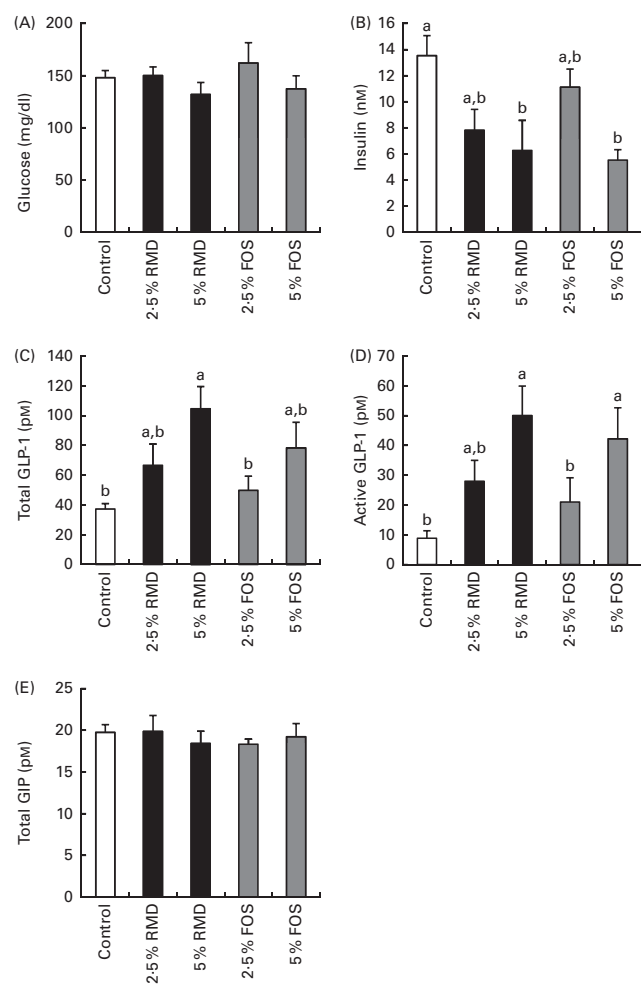


Fig. 3. Portal glucose (A), insulin (B), total glucagon-like peptide-1 (GLP-1) (C), active GLP-1 (D) and glucose-dependent insulinotropic polypeptide (GIP) (E) levels after 7 weeks of feeding the test diet. Blood samples were collected from the portal veins of rats fasted overnight after 7 weeks of feeding the test diet, and the levels of glucose, insulin, GLP-1 (total and active) and total GIP were measured in the plasma. To convert glucose in mg/dl to mmol/l, multiply by 0.0555. Values are means ($n=7-8$), with their standard errors represented by vertical bars. ^{a,b} Mean values with unlike letters were significantly different between the treatment groups ($P<0.05$; Tukey–Kramer test). RMD, resistant maltodextrin; FOS, fructo-oligosaccharides.

caecum (Fig. 4(E)) of the 2.5 and 5% RMD groups tended to be higher than that of the control group, due to the increased caecal tissue weight (Table 2). In contrast, the FOS groups had similar or slightly lower GLP-1 content in the whole caecum compared with the control group.

Although the jejunum is not a major source of GLP-1 production, proglucagon expression levels were decreased in this tissue by the RMD and FOS treatments (Fig. 5(A)). In contrast, the RMD and FOS treatments increased proglucagon expression levels in the caecum ($P=0.025$ for the 2.5% RMD group, $P<0.001$ for the 5.0% RMD group and the 5.0% FOS group *v.* the control group, respectively) and colon ($P=0.007$ for the 5.0% RMD group and $P=0.036$ for the 5.0% FOS group *v.* the control group, respectively) in a dose-dependent manner (Fig. 5(C) and (D)).

GLP-1 secretion was markedly increased by ZeinH ($P<0.001$), a dietary peptide used as a positive control⁽²⁷⁾. RMD at 20 mM induced a significant increase in GLP-1 secretion ($P<0.001$; Fig. 6), whereas FOS at 20 mM had only a mild ($P=0.097$) effect. Both RMD and FOS at concentrations lower than 20 mM did not induce GLP-1 secretion.

To examine its acute effect on GLP-1 secretion *in vivo*, RMD was orally administered to fasted rats in a separate experiment. Single oral administration of RMD gradually increased plasma GLP-1 levels, with significant elevations observed at 150 min ($P=0.040$) and 180 min ($P=0.022$), compared with the basal (0 min) value (Fig. 7). In contrast, (digestible) maltodextrin, which has a similar molecular weight as RMD, had little effect on plasma GLP-1 levels. ZeinH immediately (15–30 min) increased plasma GLP-1 levels ($P=0.040$ for 15 min and $P=0.004$ for 30 min, respectively), compared with the basal (0 min) value, followed by gradual and

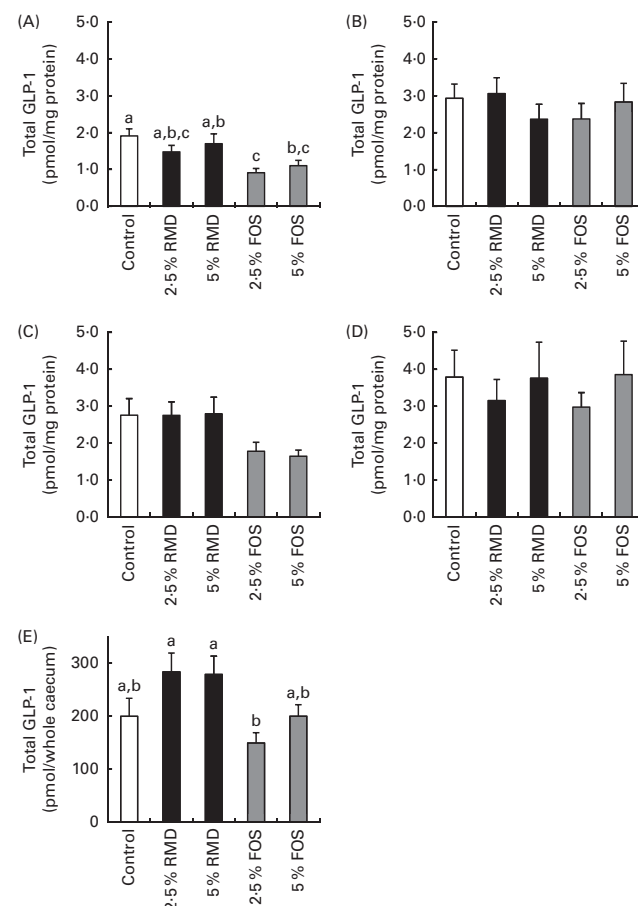


Fig. 4. Intestinal glucagon-like peptide-1 (GLP-1) concentrations after 7 weeks of feeding the test diet. Intestinal tissue samples were collected from the jejunum (A), ileum (B), caecum (C) and colon (D) of each rat. After acid–ethanol extraction, GLP-1 and total protein concentrations were measured. GLP-1 concentration was corrected according to the total protein content. GLP-1 content in the whole caecum (E) was calculated on the basis of the tissue weight of the whole caecum (Table 2). Values are means ($n=7-8$), with their standard errors represented by vertical bars. ^{a,b,c} Mean values with unlike letters were significantly different between the treatment groups ($P<0.05$; Tukey–Kramer test). RMD, resistant maltodextrin; FOS, fructo-oligosaccharides.

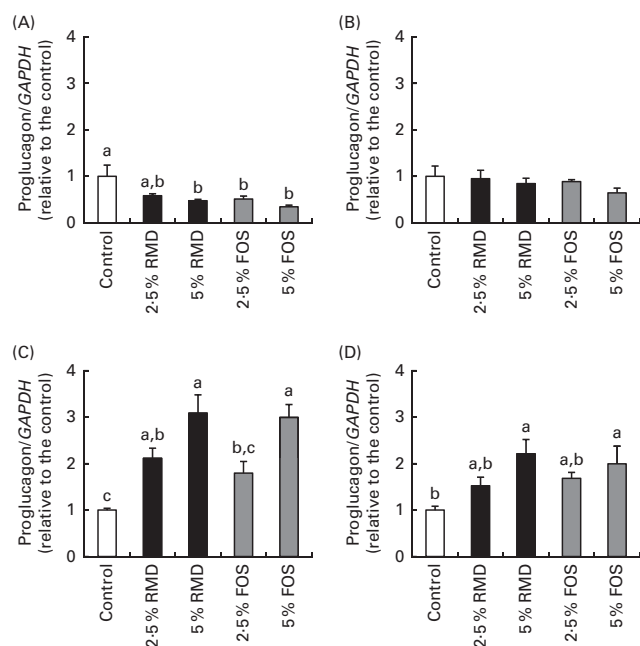


Fig. 5. Proglucagon mRNA expression levels in the intestinal mucosa. Intestinal mucosa samples were scraped from the jejunum (A), ileum (B), caecum (C) and colon (D). Total RNA was used for real-time PCR analysis. Proglucagon mRNA expression levels were normalised to those of *GAPDH* (glyceraldehyde 3-phosphate dehydrogenase), and data are expressed as relative changes to the control group. Values are means ($n = 7-8$), with their standard errors represented by vertical bars. ^{a,b,c} Mean values with unlike letters were significantly different between the treatment groups ($P < 0.05$; Tukey-Kramer test). RMD, resistant maltodextrin; FOS, fructo-oligosaccharides.

significant elevations at 150 min ($P = 0.013$) and 180 min ($P = 0.005$), compared with the basal value.

Discussion

Endogenous GLP-1 is a potential therapeutic target to improve glucose homeostasis, and possibly to prevent glucose intolerance. Although postprandial luminal nutrient-triggered GLP-1 secretion is well understood, studies related to the regulation of fasting GLP-1 levels by dietary factors are limited. In the present study, we examined whether supplementation of water-soluble non-digestible saccharides RMD and FOS in a standard diet affected fasting GLP-1 levels, intestinal GLP-1 production and glucose tolerance in normal rats.

Basal GLP-1 levels after 6 and 7 weeks of feeding the test diet were higher in the 5% RMD group than those in the control group (Figs. 1(D) and 3(C) and (D)). Because rats were fasted overnight, elevated GLP-1 levels could reflect enhanced spontaneous GLP-1 secretion, rather than induced by the remaining chyme in the intestinal lumen. The elevation of portal GLP-1 levels, with no change in GIP levels, in both the RMD and FOS groups indicates that the effect of these non-digestible saccharides was selective for GLP-1, but not for both the incretins.

Improved glucose tolerance was observed in the RMD groups, as indicated by the IPGTT (Fig. 2). Consistent with this finding, fasting insulin levels were reduced in the RMD groups (Fig. 3). In the present study, the IPGTT was

conducted as a glucose tolerance test, rather than as the conventional oral glucose tolerance test, because an oral glucose load could stimulate GLP-1 secretion, and the glycaemic response, after an oral glucose load, could be affected by the gastric emptying rate of the glucose solution and glucose absorption in the small intestine⁽²⁸⁾. The lower glycaemic response, shown in the present study, did not involve either reduced intestinal glucose absorption or luminal glucose-induced GLP-1 secretion. This could rather be due to improved insulin sensitivity in the RMD group. As GLP-1 receptor agonists improve glucose tolerance, when used as a treatment for type 2 diabetes^(3,4), enhanced basal GLP-1 levels could have contributed to the observed improvement in glucose tolerance in the present study.

GLP-1 content in the whole caecum was highest in the RMD groups (Fig. 4(E)), due to the increased caecal tissue weight (Table 2). This could partially explain why the highest plasma GLP-1 levels were observed in the 5% RMD group (Figs. 1 and 3). Proglucagon mRNA expression was markedly increased by the RMD and FOS treatments (Fig. 5), but such changes were not directly reflected by GLP-1 concentrations in the caecum and colon. Previous studies have demonstrated that GLP-1 content in the distal intestine was increased by inulin-type fructans, together with increased proglucagon mRNA expression^(17,18,25). However, in these studies, fibre contents in the diet were higher (10%) than those used in the present study (5%). Possibly, RMD at 5% was not sufficient to increase GLP-1 content in each L cell or to increase L-cell numbers. Another mechanism such as post-translational regulation might be involved in the increment of tissue GLP-1 content. Prohormone convertase 1 (PC1) plays an important role in the liberation of GLP-1 from proglucagon protein in enteroendocrine L cells. It has previously been demonstrated that oligofructose treatment increased GLP-1 content and *PC1* mRNA level in the colon of diabetic rats⁽¹⁸⁾. Interestingly,

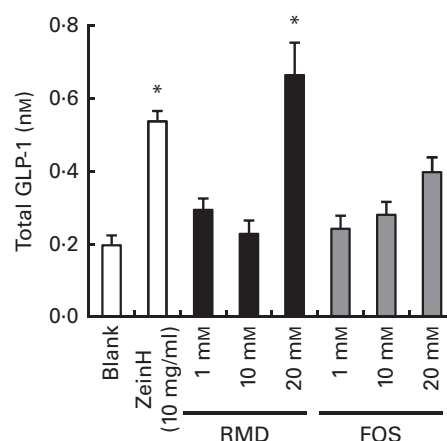


Fig. 6. Glucagon-like peptide-1 (GLP-1) secretion in glucagon-like peptide-1-producing cell line (GLUTag cells). Enterioendocrine GLUTag cells were exposed to various concentrations (1–20 mM) of resistant maltodextrin (RMD) or fructo-oligosaccharides (FOS) for 60 min. GLP-1 concentration in the supernatant was measured by ELISA. Values are means ($n = 8-20$), with their standard errors represented by vertical bars. *Mean value was significantly different from that of the control (blank) treatment ($P < 0.05$; Tukey-Kramer test).

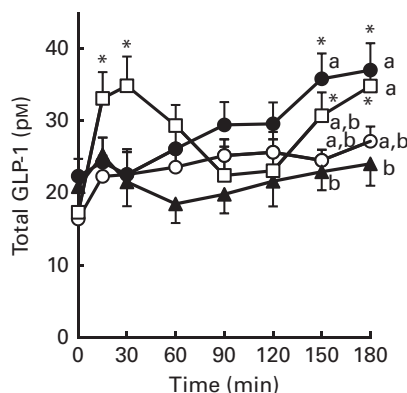


Fig. 7. Glucagon-like peptide-1 (GLP-1) secretion in response to single oral administration of resistant maltodextrin (RMD) in conscious rats. Saline (8 ml/kg, control; ○) or 2 g/kg of either RMD (●), maltodextrin (▲) or ZeinH (□) were orally administered to the fasted rats. Blood samples were collected from the jugular vein before and after oral administration. Plasma total GLP-1 concentrations were measured by ELISA. Two-way ANOVA *P* values were <0.01 for time and treatment, and <0.068 for the interaction between time and treatment. Values are means (*n* 6–8), with their standard errors represented by vertical bars. * Mean value was significantly different from that of the basal (0 min) values within each treatment group (*P* < 0.05; Dunnett's test). ^{a,b,c} Mean values with unlike letters were significantly different between the treatment groups at the same time point (*P* < 0.05; Tukey–Kramer test).

GLP-1 concentration and proglucagon mRNA expression level in the jejunum were significantly reduced in the FOS groups, and slightly reduced in the RMD groups (Fig. 5(A)). Similarly, a previous study has demonstrated that GLP-1 content in obese mice was decreased in the upper small intestine, but increased in the lower small intestine and colon, by chronic ingestion of voglibose, an α -glucosidase inhibitor⁽²⁹⁾. The mechanism of the reduction in upper small-intestinal GLP-1 production is totally unknown. However, these changes appear to have a relatively small impact on plasma GLP-1 levels because the jejunum is not a major source of GLP-1 production.

In vitro experiments, using enteroendocrine GLUTag cells, suggest that RMD directly stimulates GLP-1 secretion in L cells in the intestine (Fig. 6). Because the same concentration of FOS failed to increase the secretion, these results cannot be attributed to the osmotic effects. Previous studies using

GLUTag cells have demonstrated that potent GLP-1 secretion by glucose at 10 mM^(9,30–32), and the luminal concentration of RMD, can be estimated at about 12.5–25 mM (25–50 mg/ml), when rats consumed a 5–10 g diet, after fasting⁽³³⁾. Therefore, the concentration used in the present study can also be applied in the intestinal lumen. The direct effects of dietary fibres on GLP-1 secretion have not been demonstrated previously. RMD may be the first fibre having such a direct effect on GLP-1 secretion. RMD is composed of glucose polymers having not only 1–4 and 1–6 linkages, but also 1–2 and 1–3 linkages with a branching structure. FOS is mainly composed of 1-kestose (glucosyl-fructosyl-fructose), nystose (glucosyl-fructosyl-fructosyl-fructose) and fructofuranosylmaltose (glucosyl-fructosyl-fructosyl-fructose), all of which have a linear structure. As glucose, but not sucrose, is well known to stimulate directly GLP-1 secretion in L cells^(34,35), glucose-related or branching structure in RMD might be recognised by cells via pattern recognition receptor(s) such as Toll-like receptor(s), or orphan G protein-coupled receptor(s), that trigger GLP-1 secretion. For example, although FOS was not effective in GLUTag cells in the present study, a recent study has demonstrated that Toll-like receptor 2 is activated by β 2 $\rightarrow$ 1-fructans in the intestinal epithelium⁽³⁶⁾. Because RMD does not provide any taste, the sweet taste receptor (T1R2/T1R3) is unlikely to be involved in the stimulatory effect of RMD on GLP-1 secretion⁽³⁴⁾.

In the single oral administration experiment, RMD gradually increased plasma GLP-1 levels throughout the experimental period (Fig. 7). Because RMD is resistant to luminal digestion, it should be gradually accumulated in the lower intestine after single oral administration. Therefore, GLP-1 secretion was likely to be caused by the direct action of RMD, gradually concentrated in the middle–distal small intestine. Further studies are needed to elucidate the molecular mechanisms by which RMD stimulates GLP-1 secretion. The dietary protein hydrolysate ZeinH induced biphasic GLP-1 secretion, probably reflecting indirect and direct stimulation of GLP-1-producing cells^(27,37). The first phase was induced conceivably by indirect stimulation, triggered in the upper small intestine via the vagal pathway, and the second by a direct action in the lower small intestine⁽²⁷⁾. Zein protein is an ethanol-soluble prolamin fraction of maize protein, and well known as a

Table 2. Body weight, food intake, adipose weight, caecal weight and caecal content pH after 7 weeks of feeding the test diet (Mean values with their standard errors, *n* 7–8)

	Control		2.5 % RMD		5 % RMD		2.5 % FOS		5 % FOS		<i>P</i> (ANOVA)
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	
Body weight (g)	424.1	15.5	440.3	8.5	438.3	9.3	428.3	7.6	437.6	8.2	0.753
Daily food intake (g/d)	22.3	0.7	22.1	0.5	21.6	0.3	22.0	0.4	22.1	0.4	0.907
Mesenteric fat (g)	6.47	0.72	5.53	0.44	5.40	0.51	5.30	0.38	5.37	0.29	0.432
Epididymal fat (g)	9.48	0.93	9.42	0.99	8.56	0.61	8.75	0.82	8.46	0.47	0.843
Retroperitoneal fat (g)	11.91	0.80	11.84	1.19	11.30	0.96	10.95	0.48	10.36	0.64	0.693
Caecal tissue (g)	1.01 ^c	0.03	1.49 ^{a,b}	0.06	1.71 ^a	0.09	1.32 ^{b,c}	0.04	1.78 ^a	0.15	<0.001
Caecal content (g)	2.97 ^b	0.34	6.21 ^a	0.98	6.70 ^a	1.00	4.04 ^{a,b}	0.62	4.96 ^{a,b}	0.52	0.006
Caecal content pH	7.68 ^a	0.05	7.24 ^{b,c}	0.09	7.00 ^c	0.08	7.46 ^{a,b}	0.08	7.24 ^{b,c}	0.11	<0.001

RMD, resistant maltodextrin; FOS, fructo-oligosaccharides.

^{a,b,c} Mean values with unlike superscript letters were significantly different between the treatment groups (*P* < 0.05; Tukey–Kramer test).

low-digestible protein⁽³⁸⁾. ZeinH was prepared in our previous study from zein protein by *in vitro* papain digestion, and further *in vitro* digestion by pancreatin, which did not abolish its GLP-1-releasing effect⁽⁷⁾. This supports the notion that the second phase of GLP-1 secretion was caused by the direct action of ZeinH-derived peptide fragments that remained and accumulated in the lower small intestine. In addition, the differential response of GLP-1 secretion to ZeinH and RMD raises the interesting possibility that the induction of GLP-1 secretion in the upper small intestine is sensitive to dietary peptides, but not to RMD. The direct and acute effect of RMD on GLP-1 secretion, as shown in Figs. 6 and 7, could have enhanced the daily postprandial GLP-1 secretory response in rats fed the RMD-supplemented diet, compared with those fed the control or FOS-supplemented diet.

Both the RMD and FOS diets lowered luminal pH in the caecum, suggesting the enhancement of gut fermentation by continuous feeding of these fibres. This observation is consistent with previous reports, and a recent review has concluded that the gut fermentation of non-digestible carbohydrates is associated with the secretion of GLP-1⁽³⁹⁾. SCFA produced by the gut microbiota stimulate GLP-1 secretion and production^(22,40). The decreased pH of the caecal content in the RMD and FOS groups suggests that SCFA production is increased by enhanced gut fermentation^(15,25,41,42). Thus, SCFA production was likely to be increased in the caecum by RMD and FOS, which could stimulate GLP-1 production and secretion. Previous reports have demonstrated the promotive effect of non-digestible saccharides (e.g. inulin, FOS and resistant starch) on luminal fermentation, and GLP-1 production in animal and human studies^(39,43). In animal studies, these saccharides have been supplemented in the diet at levels of 9–30%, which are much higher than those in the present study (2.5–5%). It is expected that dietary RMD exerts promotive effects on GLP-1 and glycaemia at a relatively lower dose compared with other non-digestible saccharides, with less risk of causing diarrhoea⁽⁴⁴⁾. Further studies are necessary to investigate the effect of RMD on GLP-1 secretion/production in obese and diabetic models as well as in human subjects. Thus, the direct and indirect (through increased gut microbial fermentation) effects of RMD on both GLP-1 secretion and production possibly improve glucose tolerance (Fig. 2) through multiple action of GLP-1⁽²⁾.

In summary, rats fed a normal diet containing 5% RMD for 6 weeks showed elevated fasting GLP-1 levels, and a suppressed glycaemic response as determined by the IPGTT. Fasting GLP-1 levels in the portal vein were also elevated, and the insulin level was reduced in the 5% RMD group. Enhanced gut fermentation was suggested by the reduction of luminal pH in the caecum of rats treated with RMD and FOS, but a significant increase in GLP-1 content was observed in the RMD group only. Furthermore, RMD directly stimulated GLP-1 secretion in the enteroendocrine cell model, and single oral administration of RMD induced GLP-1 secretion in conscious rats. These results demonstrate that stimulatory effects of RMD, a soluble dietary fibre, on GLP-1 secretion and production are caused by its indirect (enhanced gut fermentation) and direct effects on GLP-1-producing cells.

Ingestion of RMD can be a novel strategy to increase endogenous GLP-1, which probably contributes to the prevention of glucose intolerance.

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The authors declare that there are no conflicts of interest, although Y. K. and S. K. are employees of Matsutani Chemical Industry.

References

1. Cho YM, Fujita Y & Kieffer TJ (2014) Glucagon-like peptide-1: glucose homeostasis and beyond. *Annu Rev Physiol* **76**, 535–559.
2. Kim YO & Schuppan D (2012) When GLP-1 hits the liver: a novel approach for insulin resistance and NASH. *Am J Physiol Gastrointest Liver Physiol* **302**, G759–G761.
3. Drucker DJ, Dritselis A & Kirkpatrick P (2010) Liraglutide. *Nat Rev Drug Discov* **9**, 267–268.
4. Madsbad S, Kielgast U, Asmar M, *et al.* (2011) An overview of once-weekly glucagon-like peptide-1 receptor agonists – available efficacy and safety data and perspectives for the future. *Diabetes Obes Metab* **13**, 394–407.
5. Yazbeck R, Howarth GS & Abbott CA (2009) Dipeptidyl peptidase inhibitors, an emerging drug class for inflammatory disease? *Trends Pharmacol Sci* **30**, 600–607.
6. Mochida T, Hira T & Hara H (2010) The corn protein, zein hydrolysate, administered into the ileum attenuates hyperglycemia via its dual action on glucagon-like peptide-1 secretion and dipeptidyl peptidase-IV activity in rats. *Endocrinology* **151**, 3095–3104.
7. Higuchi N, Hira T, Yamada N, *et al.* (2013) Oral administration of corn zein hydrolysate stimulates GLP-1 and GIP secretion and improves glucose tolerance in male normal rats and Goto–Kakizaki rats. *Endocrinology* **154**, 3089–3098.
8. Jakubowicz D, Froy O, Ahrén B, *et al.* (2014) Incretin, insulinotropic and glucose-lowering effects of whey protein pre-load in type 2 diabetes: a randomised clinical trial. *Diabetologia* **57**, 1807–1811.
9. Reimann F, Williams L, da Silva Xavier G, *et al.* (2004) Glutamine potently stimulates glucagon-like peptide-1 secretion from GLUTag cells. *Diabetologia* **47**, 1592–1601.
10. Greenfield JR, Farooqi IS, Keogh JM, *et al.* (2009) Oral glutamine increases circulating glucagon-like peptide 1, glucagon, and insulin concentrations in lean, obese, and type 2 diabetic subjects. *Am J Clin Nutr* **89**, 106–113.
11. Baer DJ, Stote KS, Henderson T, *et al.* (2014) The metabolizable energy of dietary resistant maltodextrin is variable and alters fecal microbiota composition in adult men. *J Nutr* **144**, 1023–1029.
12. Livesey G & Tagami H (2009) Interventions to lower the glycemic response to carbohydrate foods with a

- low-viscosity fiber (resistant maltodextrin): meta-analysis of randomized controlled trials. *Am J Clin Nutr* **89**, 114–125.
13. Hashizume C, Kishimoto Y, Kanahori S, *et al.* (2012) Improvement effect of resistant maltodextrin in humans with metabolic syndrome by continuous administration. *J Nutr Sci Vitaminol (Tokyo)* **58**, 423–430.
 14. Kishimoto Y, Oga H, Tagami H, *et al.* (2007) Suppressive effect of resistant maltodextrin on postprandial blood triacylglycerol elevation. *Eur J Nutr* **46**, 133–138.
 15. Miyazato S, Nakagawa C, Kishimoto Y, *et al.* (2010) Promotive effects of resistant maltodextrin on apparent absorption of calcium, magnesium, iron and zinc in rats. *Eur J Nutr* **49**, 165–171.
 16. Fastinger ND, Karr-Lilienthal LK, Spears JK, *et al.* (2008) A novel resistant maltodextrin alters gastrointestinal tolerance factors, fecal characteristics, and fecal microbiota in healthy adult humans. *J Am Coll Nutr* **27**, 356–366.
 17. Cani PD, Dewever C & Delzenne NM (2004) Inulin-type fructans modulate gastrointestinal peptides involved in appetite regulation (glucagon-like peptide-1 and ghrelin) in rats. *Br J Nutr* **92**, 521–526.
 18. Cani PD, Daubioul CA, Reusens B, *et al.* (2005) Involvement of endogenous glucagon-like peptide-1 (7–36) amide on glycaemia-lowering effect of oligofructose in streptozotocin-treated rats. *J Endocrinol* **185**, 457–465.
 19. Delzenne NM, Cani PD, Daubioul C, *et al.* (2005) Impact of inulin and oligofructose on gastrointestinal peptides. *Br J Nutr* **93**, Suppl. 1, S157–S161.
 20. Reimer RA & Russell JC (2008) Glucose tolerance, lipids, and GLP-1 secretion in JCR:LA-cp rats fed a high protein fiber diet. *Obesity (Silver Spring)* **16**, 40–46.
 21. Zhou J, Martin RJ, Tulley RT, *et al.* (2008) Dietary resistant starch upregulates total GLP-1 and PYY in a sustained day-long manner through fermentation in rodents. *Am J Physiol Endocrinol Metab* **295**, E1160–E1166.
 22. Tolhurst G, Heffron H, Lam YS, *et al.* (2012) Short-chain fatty acids stimulate glucagon-like peptide-1 secretion via the G-protein-coupled receptor FFAR2. *Diabetes* **61**, 364–371.
 23. Reeves PG (1997) Components of the AIN-93 diets as improvements in the AIN-76A diet. *J Nutr* **127**, 838S–841S.
 24. Cacho J, Sevillano J, de Castro J, *et al.* (2008) Validation of simple indexes to assess insulin sensitivity during pregnancy in Wistar and Sprague–Dawley rats. *Am J Physiol Endocrinol Metab* **295**, E1269–E1276.
 25. Cani PD, Hoste S, Guiot Y, *et al.* (2007) Dietary non-digestible carbohydrates promote L-cell differentiation in the proximal colon of rats. *Br J Nutr* **98**, 32–37.
 26. Iwaya H, Lee JS, Yamagishi S, *et al.* (2012) The delay in the development of experimental colitis from isomaltosyloligosaccharides in rats is dependent on the degree of polymerization. *PLOS ONE* **7**, e50658.
 27. Hira T, Mochida T, Miyashita K, *et al.* (2009) GLP-1 secretion is enhanced directly in the ileum but indirectly in the duodenum by a newly identified potent stimulator, zein hydrolysate, in rats. *Am J Physiol Gastrointest Liver Physiol* **297**, G663–G671.
 28. Muramatsu M, Hira T, Mitsunaga A, *et al.* (2014) Activation of the gut calcium-sensing receptor by peptide agonists reduces rapid elevation of plasma glucose in response to oral glucose load in rats. *Am J Physiol Gastrointest Liver Physiol* **306**, G1099–G1107.
 29. Moritoh Y, Takeuchi K & Hazama M (2009) Chronic administration of voglibose, an α -glucosidase inhibitor, increases active glucagon-like peptide-1 levels by increasing its secretion and decreasing dipeptidyl peptidase-4 activity in ob/ob mice. *J Pharmacol Exp Ther* **329**, 669–676.
 30. Parker HE, Adriaenssens A, Rogers G, *et al.* (2012) Predominant role of active versus facilitative glucose transport for glucagon-like peptide-1 secretion. *Diabetologia* **55**, 2445–2455.
 31. Gribble FM, Williams L, Simpson AK, *et al.* (2003) A novel glucose-sensing mechanism contributing to glucagon-like peptide-1 secretion from the GLUTag cell line. *Diabetes* **52**, 1147–1154.
 32. Hira T, Muramatsu M, Okuno M, *et al.* (2011) GLP-1 secretion in response to oral and luminal palatinose (isomaltulose) in rats. *J Nutr Sci Vitaminol (Tokyo)* **57**, 30–35.
 33. Chen W, Hira T, Nakajima S, *et al.* (2012) Suppressive effect on food intake of a potato extract (Potein[®]) involving cholecystokinin release in rats. *Biosci Biotechnol Biochem* **76**, 1104–1109.
 34. Kokrashvili Z, Mosinger B & Margolskee RF (2009) T1r3 and α -gustducin in gut regulate secretion of glucagon-like peptide-1. *Ann N Y Acad Sci* **1170**, 91–94.
 35. Shirazi-Beechey SP, Daly K, Al-Rammahi M, *et al.* (2014) Role of nutrient-sensing taste 1 receptor (T1R) family members in gastrointestinal chemosensing. *Br J Nutr* **111**, Suppl. 1, S8–S15.
 36. Vogt LM, Meyer D, Pullens G, *et al.* (2014) Toll-like receptor 2 activation by β 2 $\rightarrow$ 1-fructans protects barrier function of T84 human intestinal epithelial cells in a chain length-dependent manner. *J Nutr* **144**, 1002–1008.
 37. Rocca AS & Brubaker PL (1999) Role of the vagus nerve in mediating proximal nutrient-induced glucagon-like peptide-1 secretion. *Endocrinology* **140**, 1687–1694.
 38. Sarwar G (1997) The protein digestibility-corrected amino acid score method overestimates quality of proteins containing antinutritional factors and of poorly digestible proteins supplemented with limiting amino acids in rats. *J Nutr* **127**, 758–764.
 39. Everard A & Cani PD (2014) Gut microbiota and GLP-1. *Rev Endocr Metab Disord* **15**, 189–196.
 40. Nöhr MK, Pedersen MH, Gille A, *et al.* (2013) GPR41/FFAR3 and GPR43/FFAR2 as cosensors for short-chain fatty acids in enteroendocrine cells vs FFAR3 in enteric neurons and FFAR2 in enteric leukocytes. *Endocrinology* **154**, 3552–3564.
 41. Matsukawa N, Matsumoto M, Shinoki A, *et al.* (2009) Nondigestible saccharides suppress the bacterial degradation of quercetin aglycone in the large intestine and enhance the bioavailability of quercetin glucoside in rats. *J Agric Food Chem* **57**, 9462–9468.
 42. Campbell JM, Fahey GC & Wolf BW (1997) Selected indigestible oligosaccharides affect large bowel mass, cecal and fecal short-chain fatty acids, pH and microflora in rats. *J Nutr* **127**, 130–136.
 43. Mansour A, Hosseini S, Larijani B, *et al.* (2013) Nutrients related to GLP1 secretory responses. *Nutrition* **29**, 813–820.
 44. Kishimoto Y, Kanahori S, Sakano K, *et al.* (2013) The maximum single dose of resistant maltodextrin that does not cause diarrhea in humans. *J Nutr Sci Vitaminol (Tokyo)* **59**, 352–357.